Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 28, SEC. F. DECEMBER, 1950 NUMBER 12

THE EFFECT OF CITRIC ACID UPON THE OXIDATION OF PEANUT OIL AND OF THE METHYL ESTERS DERIVED FROM PEANUT OIL[1]

BY H. W. LEMON, RUTH M. KNAPP, AND A. H. ALLMAN

Abstract

Addition of 0.005–0.500% citric acid to refined peanut oil caused a marked retardation of the oxygen absorption by the oil at 100°C. Conversely, oxygen absorption was accelerated by the addition of from 0.11 to 10.00 p.p.m. iron in the form of iron stearate. When iron and citric acid were added together, the accelerating effect of iron on oxidation was overcome when the molar ratio of citric acid to iron was about 23: 1; with higher concentrations of citric acid, oxidation was retarded still further.

With distilled methyl esters of the fatty acids of peanut oil, further purified by passage through a column containing activated carbon, citric acid addition alone had only a small effect. Addition of iron caused acceleration of oxygen absorption which was compensated for by suitable addition of citric acid. Addition of hydroquinone caused a retardation of oxygen absorption, and still further retardation occurred when citric acid and hydroquinone were added together. It was concluded that citric acid has the properties of both a metal deactivator and a synergist, but does not in itself function as an antioxidant.

Introduction

Citric acid has been recognized for some time as an effective antioxidation factor (6). The early German thesis as to its effect centered around lecithin (3). It was believed that lecithin was a pro-oxidant which was bound by the addition of citric acid. The most popular theory concerning the function of citric acid in oxidation is that it acts as a synergist in conjunction with naturally occurring antioxidants (7, 10, 11, 12, 15). Olcott and Mattill (11) found that, when using ethyl esters of lard, distillation removed certain compounds upon which the action of citric acid was dependent. By putting back the distillation residue, the protective role of the organic acid was restored.

More recently citric acid has been studied because of its beneficial effect in inhibiting flavor reversion in edible fats and oils (4, 5, 9). Dutton and coworkers (4) have suggested that the protection afforded by citric acid is due, not only to its synergistic effect, but also to metal deactivation. They found the organic acid was active in a "tocopherol-free" substrate, and concluded that synergism was not the only function of the acid. The theory of metal

[1] *Manuscript received July 27, 1950.*
Contribution from the Department of Biochemistry, Ontario Research Foundation, Toronto, Ontario, with financial assistance from the National Research Council of Canada.

deactivation by citric acid has many supporters (4, 5, 9), and work is being done at the present time to establish data on the subject. According to this theory, an iron–citric complex is formed which is inactive as an oxidation catalyst. Several formulas have been suggested for this complex (2, 16).

Recently Morris and coworkers (8) suggested a possible relationship between the synergistic and metal deactivating properties of the acid, although they believed the two effects to be separate.

Evidence is presented in this paper to show (i) that citric acid is (*a*) a synergist, enhancing the action of natural antioxidants and (*b*) a metal deactivator, reducing oxidative catalysis caused by dissolved iron, and (ii) that both effects proceed independently.

Materials and Methods

The materials used in the oxygen absorption experiments were (*a*) refined peanut oil, (*b*) undistilled methyl esters of peanut oil fatty acids, (*c*) distilled methyl esters, and (*d*) distilled esters from which tocopherols had been removed.

The refined peanut oil was dehydrated by heating to 75°C. under vacuum, and was stored at 0°F. until needed for use. The peroxide values of the samples remained essentially unchanged for a year at this temperature.

The methyl esters were prepared by a method similar to that described by Wright *et al.* (18). The iodine value of the undistilled methyl esters was 91.7. A portion of the unpurified esters was twice distilled under vacuum using all-glass equipment. As a portion of the saturated esters did not distill, the iodine value of the distillate was 96.3. A quantity of the distilled esters was passed through a column of activated carbon for removal of natural antioxidants, according to the method of Dutton (4). After treatment of the esters in this way, neither tocopherol nor iron could be detected by the methods of measurement described below. The iodine value of this purified, "tocopherol-free" product was 97.6.

The tocopherol assays were made on the unsaponifiable fractions of the above samples using the method of Rawlings (13). The calibration curve was obtained using pure hydroquinone. Transmission measurements were made at 518 mμ using the Beckman quartz spectrophotometer.

Samples were also analyzed for iron. Ten grams of the sample was weighed into a silica crucible. The bulk of the oil was burned with the aid of a wick made of ashless filter paper, similar to the method described by Berl and Lunge (1), except that the platinum wire to hold the wick was found to be unnecessary for our work. Ashing was completed in a muffle furnace at 500°C. Two milliliters of hydrochloric acid was added to the ash, the crucible covered with a watch glass, and the contents refluxed for 20 min. The glass and sides of the crucible were washed with distilled water, all washings being directed

into the crucible. The contents was evaporated to near dryness, and transferred to a 25 ml. volumetric flask with distilled water. The following reagents were added in aqueous solutions: 2 ml. of 5% hydroxylamine hydrochloride, 3 ml. of 2*M* sodium acetate and 2 ml. of 0.1% α,α'-dipyridyl, and the whole made up to volume. The amount of iron present was determined with the Beckman spectrophotometer, by measuring transmission at a wave length of 520 mμ. The fundamental principles of this method of iron determination are given by Sandell (14).

The rate of autoxidation of the substrate was measured by means of a Warburg constant volume respirometer. A description of this apparatus is given by Umbreit, Burris, and Stauffer (17). The manometer fluid used was "Silicone"*. This fluid was found to have a sufficiently low vapor pressure, and was sensitive to small pressure changes which was essential when minute quantities of oxidizable material were under investigation. The fluid reservoir at the base of each manometer was connected by Tygon pressure tubing which was found to be particularly good because it was impervious to the silicone fluid. The flasks used were standard 50 ml. Pyrex Erlenmeyers to which ground glass joints were fixed to connect to the side arms of the manometers. Silicone high vacuum grease was used on the ground glass fittings because of its oxidative stability and its usefulness over a wide temperature range. A sample of approximately 0.2 gm. weighed accurately into each flask produced a thin film of oil. The manometers and flasks, connected by T-tubes were evacuated and filled with oxygen. The flasks, when in position, dipped into a constant temperature oil bath. Agitation of the flasks was accomplished by a shaker-mechanism at a rate of approximately 125 cycles per min. Oxidation of the peanut oil samples was done at 100°C. but oxidation of the methyl esters was done at 80°C. because the rate of oxygen uptake by the esters at 100°C. was too rapid. After an equilibration period of 20 min., the manometer systems were closed and readings begun. The oxygen uptake was calculated in milliliters per gram of sample according to the method of Umbreit *et al.* (17). The experimental points found on the graphs presented are the actual points obtained by measurement of gas absorption from two separate reaction flasks containing the same substrate.

Experimental

Oxygen absorption experiments were carried out to investigate the following: (*a*) the individual and combined effects of iron and citric acid upon oxidation of peanut oil, (*b*) the action of citric acid upon the oxidation of undistilled methyl esters of peanut oil fatty acids, distilled methyl esters, and "tocopherol-free" distilled methyl esters respectively, (*c*) the effect upon oxidation of adding iron and hydroquinone singly, and in the presence of citric acid, to the purified distilled methyl esters.

* *Dow Corning Corporation, Midland, Mich. Fluid No. 200. Viscosity at 25°C., 3 centistokes.*

To study the catalytic action of iron upon the oxidation of peanut oil and its esters, known quantities were dissolved in the substrate. The iron was added as iron stearate and a 100 p.p.m. iron solution was made by dissolving 0.0459 gm. in 30 gm. oil, the mixture being held under vacuum at 75°C. until dissolution was completed. Dilutions of this concentrate were made for use in Warburg oxygen absorption experiments.

Known amounts of a 20% aqueous solution of citric acid were weighed into the oil and the mixture was deodorized at 100°C. for one hour, which removed the water and incorporated the citric acid into the oil. This method of incorporation is similar to that employed by Dutton (4). When more than 0.5% citric acid was incorporated into the oil, crystallization occurred.

Hydroquinone was added to the esters to replace in part the antioxidants removed by purification of the peanut oil. It was found that the oil contained 300 μgm. of tocopherols per gram of sample. An aqueous solution of hydroquinone equivalent to 58 μgm. of tocopherols per gram of sample was added to the esters. Solution was effected by deodorizing at 100°C. for one hour. In cases where more than one of iron, citric acid, or hydroquinone were to be added to the substrate, solution of the additives by deodorization was completed simultaneously.

Results and Discussion

Fig. 1 shows the acceleration of oxygen uptake caused by varying amounts of dissolved iron added to peanut oil. No attempt has been made to establish a quantitative relationship, although the evidence strongly suggests it may exist. An acceleration of oxygen absorption resulted even when quantities

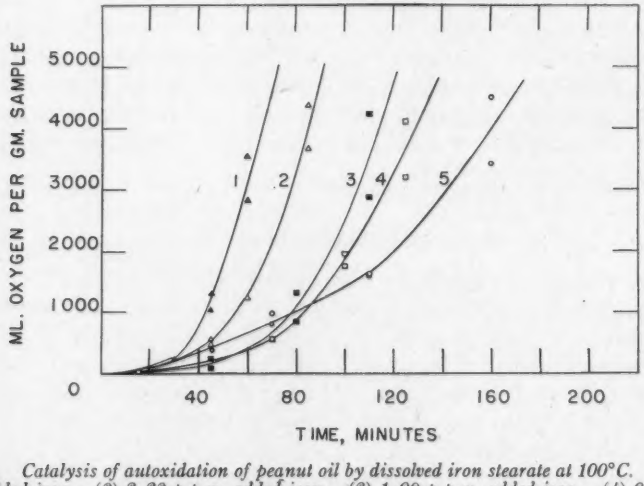

FIG. 1. *Catalysis of autoxidation of peanut oil by dissolved iron stearate at 100°C. (1) 10.01 p.p.m. added iron. (2) 3.32 p.p.m. added iron. (3) 1.09 p.p.m. added iron. (4) 0.36 p.p.m. added iron. (5) Control,– refined, dehydrated peanut oil.*

of iron as small as 0.11 p.p.m. were added to the oil. The curves for 1.09 p.p.m. (3) and 0.36 p.p.m. (4) of added iron fall below the control (5) for times below 80 min. If this is significant, as it appears to be, it means that small additions of iron caused a decrease in the rate of oxidation of the substrate in the initial phase but accelerated the last phase of the reaction. The results suggest that iron catalyzes the decomposition of hydroperoxides rather than their formation.

When 0.1% citric acid was added to refined peanut oil, the inhibition of oxygen uptake was very marked. The control sample absorbed 500 ml. oxygen per gm. of sample in 110 min., whereas the oil containing citric acid required 280 min. for the absorption of the same amount of oxygen.

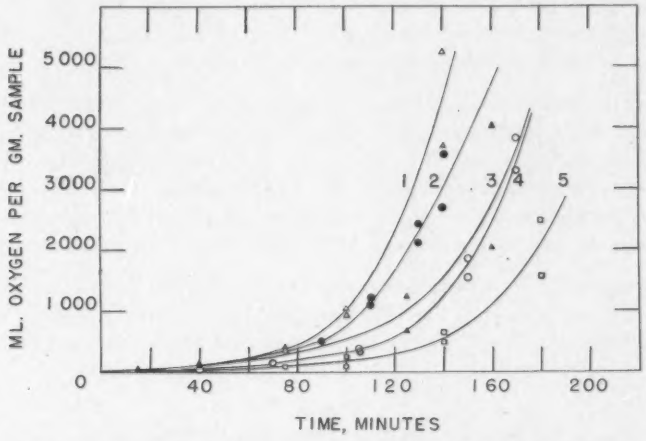

FIG. 2. *Citric acid required to compensate for catalysis caused by 3.39 p.p.m. added iron, in peanut oil oxidized at 100°C. (1) Refined peanut oil + 3.39 p.p.m. iron + 0.0075% citric acid. (2) Refined peanut oil +3.39 p.p.m. iron + 0.0150% citric acid. (3) Refined peanut oil + 3.39 p.p.m. iron + 0.0225% citric acid. (4) Refined peanut oil. (5) Refined peanut oil + 3.39 p.p.m. iron + 0.0300% citric acid.*

Fig. 2 shows the effect of citric acid concentration on the oxidation of peanut oil to which 3.39 p.p.m. iron was added. Approximately 0.0225% citric acid by weight reduced the rate of oxidation to the same rate as that of the control oil containing neither added iron nor citric acid. The rate was less than that of the control when a greater concentration was used, which indicates that the citric acid not only deactivated the iron, but also acted as either an antioxidant or a synergist with the antioxidants present in the oil. When a large excess of citric acid was used, the oxidation of peanut oil was virtually stopped. Although the oil contained some naturally occurring iron, the concentration of the latter was relatively small.

Table I gives the percentages of citric acid required to compensate for varying concentrations of iron added to peanut oil. A minimum concentra-

TABLE I

CITRIC ACID REQUIRED TO COMPENSATE FOR THE EFFECT OF ADDED IRON IN PEANUT OIL AT 100°C.

P.p.m. iron added	% citric acid required	Molar ratio iron: citric
0.33	0.0050	1:52
1.11	0.0050	1:13
1.75	0.0050	1: 8
2.00	0.0150	1:23
2.50	0.0200	1:23
3.39	0.0225	1:20
5.00	0.0400	1:23

tion of 0.005% citric acid seemed to be required for the deactivation of added iron up to a concentration of 2 p.p.m. Above this concentration the molar ratio of iron to citric acid was approximately constant.

Two experiments were designed to investigate more closely the effects of citric acid upon oxidation. If citric acid is not an antioxidant in itself it should have no inhibiting effect upon the oxidation of esters from which both metals or antioxidants have been removed. Furthermore, by addition of metals or antioxidants to such a preparation, the effect of citric acid as a metal deactivator or as a synergist with antioxidants can be studied separately.

In the first experiment the three methyl ester preparations already described were used as substrates. The iron and tocopherol contents of these esters are given in Table II. Each purification step caused a reduction in both iron and

TABLE II

REMOVAL OF ANTIOXIDANTS AND IRON FROM PEANUT OIL

Sample	P.p.m. iron	μgm. tocopherol per gram
Peanut oil	2.11	300
Crude methyl esters	0.38	150
Distilled methyl esters	0.19	42
Chromatographed distilled methyl esters	0.00	0

tocopherols, and their concentrations in the purest sample were so low that they could not be measured by the analytical methods employed. The action of citric acid upon oxidation of each of these preparations was noted and the results of this experiment are given in Fig. 3. It is evident that the more complete the removal of iron and antioxidants, the less is the inhibiting effect of citric acid. This is strong evidence that citric acid is not an antioxidant in itself.

In the second experiment the "tocopherol-free" esters were used as substrate. The effect of adding iron and hydroquinone, both singly and in the presence of

a large excess of citric acid, to these esters is shown in Fig. 4. The acceleration of oxidation by iron was compensated for by the citric acid which reduced the rate of oxidation to that of the control sample. The acid had only a slight inhibitory action upon the control sample. The citric acid definitely enhanced the antioxygenic function of hydroquinone.

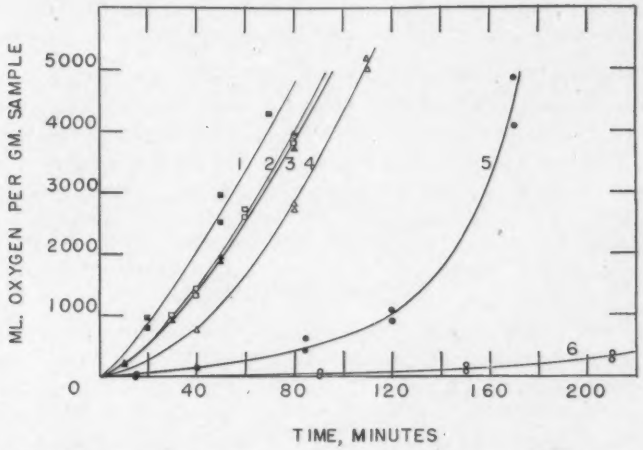

FIG. 3. *The effect of purifying the substrate upon the inhibitory action of citric acid on oxidation at 80°C. (1) "Tocopherol-free" distilled methyl esters of peanut oil. (2) "tocopherol-free" distilled methyl esters + 0.5% citric acid. (3) Distilled methyl esters. (4) Distilled methyl esters + 0.5% citric acid. (5) Undistilled methyl esters. (6) Undistilled methyl esters + 0.5% citric acid.*

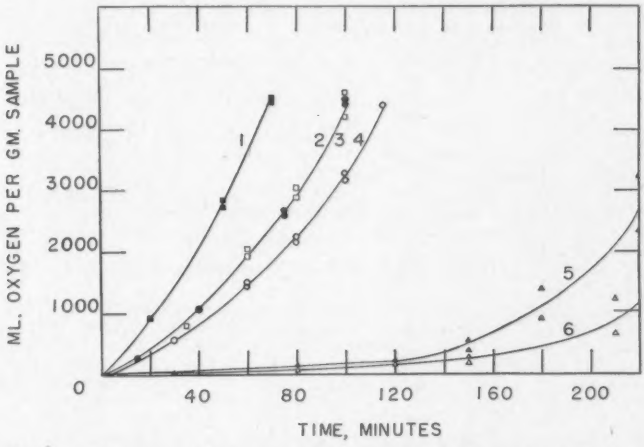

FIG. 4. *Citric acid as a metal deactivator and synergist in "tocopherol-free" methyl esters oxidized at 80°C. (1) Purified esters + 5 p.p.m. added iron. (2)-●- Purified esters + 5 p.p.m. added iron + 0.5% citric acid. (3) -□- Control,- purified esters. (4) Purified esters + 0.5% citric acid. (5) Purified esters + hydroquinone equivalent to 58 μgm. tocopherol per gm. (6) Purified esters + hydroquinone + 0.5% citric acid.*

Conclusions

The oxidation of refined peanut oil is retarded by the addition of citric acid, and is catalyzed by the addition of iron in a soluble form. The catalytic effect of added iron can be neutralized by suitable addition of citric acid to the oil. The inhibiting effect of citric acid upon the oxidation of peanut oil is undoubtedly due both to a deactivation of the iron occurring naturally in the oil, and to a synergistic effect with the antioxidants that are also present.

Citric acid has little effect upon the oxidation of a substrate containing neither iron nor antioxidants. The addition of iron to such a substrate results in catalysis of oxidation, and this effect can be neutralized by the addition of citric acid. When hydroquinone is added to the purified substrate, the rate of oxidation is retarded, and further retardation occurs when citric acid is added as well. Citric acid, therefore, has a dual action, metal deactivation and synergism.

Acknowledgments

The authors wish to acknowledge the able technical assistance of G. A. Weaver, V. R. Thomson, and Anne G. Tait.

References

1. Berl, E. and Lunge, G. Chemisch-technische Untersuchungsmethoden. Verlag von Julius Springer. Berlin. 1933.
2. Bobtelsky, M. and Jordan, J. J. Am. Chem. Soc. 69: 2286-2290. 1947.
3. Dutton, H. J., Moser, Helen A., and Cowan, J. C. J. Am. Oil Chemists' Soc. 24: 261-264. 1947.
4. Dutton, H. J., Schwab, A. W., Moser, Helen A., and Cowan, J. C. J. Am. Oil Chemists' Soc. 25: 385-388. 1948.
5. Dutton, H. J., Schwab, A. W., Moser, Helen A., and Cowan, J. C. J. Am. Oil Chemists' Soc. 26: 441-444. 1949.
6. Greenbank, G. R. and Holm, G. E. Ind. Eng. Chem. 26: 243-245. 1934.
7. Lips, A. and McFarlane, W. D. Oil and Soap, 20: 193-196. 1943.
8. Morris, S. G., Meyers, J. S., Jr., Kip, Mary L., and Riemenschneider, R. W. J. Am. Oil Chemists' Soc. 27: 105-107. 1950.
9. Moser, Helen A., Jaeger, Carol M., Cowan, J. C., and Dutton, H. J. J. Am. Oil Chemists' Soc. 24: 291-296. 1947.
10. Olcott, H. S. Oil and Soap, 18: 77-80. 1941.
11. Olcott, H. S. and Mattill, H. A. J. Am. Chemists' Soc. 58: 2204-2208. 1936.
12. Olcott, H. S. and Mattill, H. A. Chem. Revs. 29: 257-268. 1941.
13. Rawlings, H. W., Kuhrt, N. H., and Baxter, J. G. J. Am. Oil Chemists' Soc. 25: 24-26. 1948.
14. Sandell, E. B. Colorimetric determination of traces of metals. Interscience Publishers Inc., New York. 1944.
15. Stirton, A. J., Turer, J., and Riemenschneider, R. W. Oil and Soap, 22: 81-83. 1945.
16. Taichi, Harada. Sci. Papers Inst. Phys. Chem. Research, Tokyo, 41: 68-72. 1943. Chem. Abstracts, 41: 6206A. 1947.
17. Umbreit, W. W., Burris, R. H., and Stauffer, J. F. Manometric techniques and related methods for the study of tissue metabolism. Burgess Publishing Co., Minneapolis, Minn. 1946.
18. Wright, H. J., Segur, J. B., Clark, H. V., Coburn, S. K., Langdon, E. E., and Du Puis, R. N. Oil and Soap, 21: 145-148. 1944.

THE WATER-REPELLENCY OF SILICONE-TREATED SINTERED GLASS PLATES[1]

By M. G. Barrington, D. E. Fleet, and R. R. McLaughlin

Abstract

The hydrostatic pressures that silicone-coated sintered glass plates will withstand before permitting the passage of water are recorded, and a rough constancy in the product of rupturing pressure and porosity is indicated. Application as check-valves to pass gases but not water is suggested.

Introduction

Sintered glass plates when treated with Dow-Corning silicone fluids to render them water repellent are able to withstand quite high hydrostatic pressures (300 cm. of water) before permitting the passage of water. They will, of course, permit the relatively free passage of gas. Therefore they may be used as valves that will pass a gas but not water, within certain limits of pressures, in the same fashion as Lewis (1) used mercury in his pressure regulating apparatus for vacuum systems. This paper presents a statement of hydrostatic pressures that plates of different porosities can withstand, after various treatments.

Experimental

Pyrex sintered glass funnels of fine, medium, and coarse porosity were coated with Dow-Corning fluids DC 500 or DC 200 to render them water repellent*, and repeatedly tested for the maximum hydrostatic pressure they would withstand before permitting the passage of water. They were dried between each test, and recoated with silicone fluid between each series of tests. Water alone was used in the test apparatus illustrated in Fig. 1 for pressures below 100 cm. water, and water and mercury for higher pressures. In the latter case the auxiliary leveling bulb, E', containing water, was used for the final adjustment. The rupturing pressure recorded was that pressure at which, during a five minute interval after pressure increments of 0.2 cm. of water, the first droplet appeared on the upper side of F.

Porosities of the plates were determined by finding the air pressure necessary to force a bubble through a layer of carbon tetrachloride on the upper side of F. The apparatus illustrated in Fig. 1 was used for this, also, by a change in procedure. The porosities were then calculated from the formula†

[1] *Manuscript received in original form July 6, 1950, and as revised, August 30, 1950. Contribution from the Department of Chemical Engineering, University of Toronto, Toronto, Ont.; from a thesis presented by two of the authors (M.G.B. and D.E.F.) for the degree of B.A.Sc.*

* *Method supplied by Dow-Corning Corporation, Midland, Michigan.*

† *Supplied by Corning Glass Works, Corning, N.Y.*

Maximum pore diameter, in microns =

$$\frac{4 \times \text{surface tension of } CCl_4 \text{ in dynes/cm.} \times 760}{\text{pressure in mm. of mercury} \times 100}$$

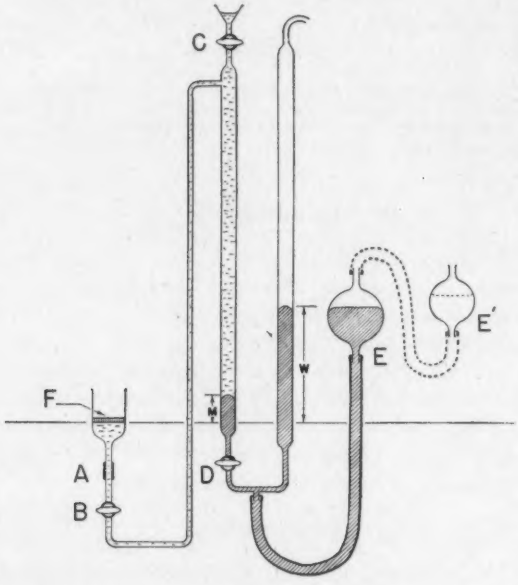

FIG. 1.

Results

Plate* number	Average rupturing pressure, cm. water		Pore diameter, microns	
	Silicone treatment		Silicone treatment	
	First	Second	First	Second
C 200	21.5	20.4	77	78
C 500	17.2	14.2	77	75
M 200	64.4	97	14.0	14.2
M 500	128	110	12.9	12.8
F 200	259	180	5.6	5.6
F 500	289	269	4.9	5.1

* *C, M, F denote the porosity; 200, 500 denote the silicone fluid.*

Comments

In general, the rupturing pressure decreases by about 2.5% for each rupturing and drying cycle. Readings taken immediately before and after the second treatment with silicone fluid indicated that the second treatment had little effect, though this is not apparent from the table as averages have been recorded.

As might be expected, there is a rough constancy in the product of rupturing pressure and pore diameter, the numbers obtained being 1655, 1324, 900, 1650, 1450, 1415, 1592, 1065, 1378, 1410, 1008, 1372.

Acknowledgments

The authors wish to acknowledge the earlier work of J. R. S. Newhouse described in his B.A.Sc. thesis (1946), and also information received from the Dow-Corning Co., and the Corning Glass Works.

Reference

1. LEWIS, F. M. J. Ind. Eng. Chem., Anal. Ed. 13: 418. 1941.

A SIMPLE SPINNER FOR ROLL TUBES USED IN ESTIMATING BACTERIAL POPULATIONS[1]

By F. H. S. Newbould

Abstract

In estimating bacterial populations by the colony count method increased accuracy is obtained by using large numbers of replicates. The high cost of Petri plates makes this difficult in many laboratories. A simple spinner is described which utilizes cheap, easily obtained screw-capped bottles to substitute for Petri plates. The method has proved satisfactory in estimating numbers of *Rhizobia*, and has resulted in significant savings in the cost of glassware and media, and in incubator space.

Introduction

It is becoming increasingly apparent, as the result of the application of statistical methods of analysis to plate counting procedures, that it is necessary to increase the number of replicate plates from each dilution if any worthwhile degree of accuracy is to be obtained. Snyder (5) has shown, as an example, that in a certain series of platings a standard error of 4.37% of the mean count provided by three plates was reduced to 2.52% by nine plates, and to 1.78% with eighteen plates. The cost of Petri plates and incubator space necessary to make counts in nine or eighteen replications is almost totally prohibitive to the majority of laboratories. It would therefore be of great benefit if some means of overcoming this economic hurdle was available.

Wilson (6) has described the use of "roll tubes" to replace Petri plates in enumerating bacteria by the colony count method. He used 6 by 5/8 in. test tubes, in which were sterilized 2 ml. amounts of agar medium. When required for use the tubes were heated to melt the agar, and then placed in a water bath at 45°C. The inoculum was added directly to the medium, the mixture gently shaken, the tube then rolled between the fingers while held at an angle under the cold water tap, the medium solidifying around the inner surface of the tube in about one minute. He reported that the counts thus obtained were approximately 5% higher than replicates obtained in Petri plates.

Snyder (5) included Wilson's technique in a survey of the relative errors of bacteriological plate count methods. He reported suggestive evidence that this method will give a higher, and therefore a presumably more accurate, count than the use of Petri plates. In his opinion, however, it was not sufficiently convenient for large scale studies.

[1] *Manuscript received July 31, 1950.*
Contribution from the Department of Bacteriology, Ontario Agricultural College, Guelph, Ont.

Julius (3) reported the use in the Laboratory of Hygiene at Utrecht of a modification of the roll tube technique which he considered to be superior to the Petri plate method. A machine was described which spun small bottles, having rubber or bakelite screw-cap closures, in which the inoculum was added to 2.7 ml. of agar medium, the spinning being continued until the inoculated medium had solidified on the sides of the bottle. These bottles gave a surface area of 19 sq. cm. and possessed the disadvantage that only small amounts of inoculum could be used. He estimated the saving of time at something less than 50%, staff 75%, and material 80–90%, as compared with the use of Petri plates.

Rowlands (4) at the National Institute for Research in Dairying in England has used a modification of the Julius machine for routine milk counts. His machine used a bottle approximately 6 cm. long and 2.5 cm. in diameter, with a neck 1.5 cm. in length, which provides a surface area of 42 sq. cm. or approximately 2/3 that of the ordinary 100 mm. Petri plates. The bottles were modified to overcome the problem of the slipping of the solidified medium, although this was in part controlled by increasing the concentration of agar in the medium. He estimated that the roll tube technique reduced the over-all cost of bacterial counts to about 20% of that using Petri plates.

It is the purpose of this paper to describe a small spinner designed to use cheap, easily obtained glass vials or bottles which have proved a satisfactory substitute for Petri plates.

Description of Spinner

The spinner (see Fig. 1) consists of a small brushless-type electric fan motor, having a speed of approximately 1100 r.p.m., to the shaft of which is attached a holder for the bottles. This holder is made from a piece of light alloy

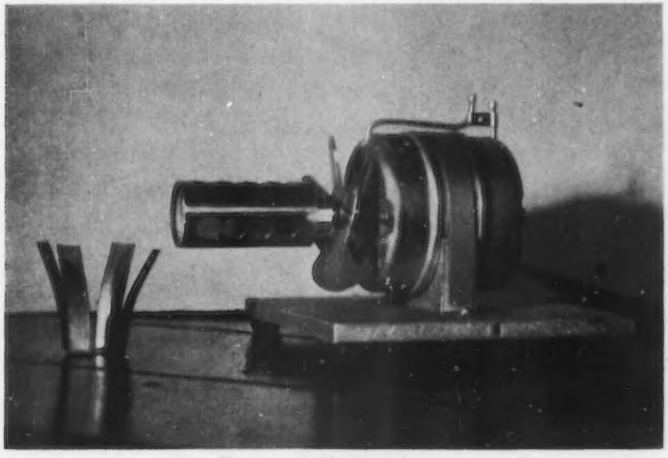

FIG. 1. *Roll tube spinner.*

tubing, one end being fitted with an adapter to connect it to the motor shaft. Four rows of four holes each are drilled in the tube to help dissipate heat from the bottle when spinning. Into the tube is pushed a spring device which serves to grip the bottle and take up small variations in bottle size. This device is shown in front of the spinner in Fig. 1, and is made from two pieces of spring brass shim stock soldered together in the form of a cross, the four arms being bent up and curved out to fit inside the tube and just long enough to fit up against a narrow ridge formed at the open end of the tube by turning out behind it a shallow groove approximately 4 mm. wide. This ridge, plainly visible in Fig. 1, serves to retain the spring device when bottles are removed from the holder. The inside dimensions of this holder, for the bottles described below, are 6 cm. long and 3 cm. in diameter, but could of course be altered to suit any other size of bottle. The motor of this spinner, being brushless, while having sufficient torque for the purpose of spinning the bottle, may be stopped by grasping the bottle holder with the fingers, thus obviating the need for a switch and the time required for stopping and starting a brush type motor. Between bottle holder and motor is mounted a small four-bladed fan, which serves to prevent overheating of the motor.

Bottles

The bottles in use in this laboratory are No. 2 capsule vials with 20 mm. screw caps, and give a surface area practically identical with those used by Rowlands. They are standard druggists' glassware except that they are made with a neck to take a 20 mm. instead of the 24 mm. cap normally supplied. This modification provides a slightly deeper shoulder which prevents the warm medium running into the cap when placed horizontally in the holder. No trouble has been encountered with slipping of the solidified medium as long as the agar (Difco granulated) content of the medium is 1.7% or higher, although 2% is being used to allow a margin of safety.

Operation

The medium, the nutrient content of which is adjusted to 6/5 times normal, is dispensed in the bottles, 5 ml. in each, and sterilized. This adjustment of nutrient content, to overcome the greater than usual dilution of the medium, results in the final mixture of medium and inoculum containing proper concentrations of nutrients. As required for use sufficient bottles are melted and placed in a water bath at 45°C. To the melted medium is added 1 ml. of inoculum, the bottle gently shaken, briefly sprayed with cold water, and placed in the spinner. Solidification takes place, even at room temperatures in excess of 90°F., in approximately one minute.

Discussion

Roll tubes have been in use in a research project of this laboratory for several months to replace Petri plates in estimating numbers of *Rhizobia*. These organisms are notoriously difficult to count (7), but, using the control

chart for colony counts developed by Hannay (2), based on the χ^2 method for checking the accuracy of these counts (1), it has been possible in approximately 150 series of replicates to keep within the probability limits of 0.05 to 0.95 the required nine times out of ten.

From the economic standpoint the savings obtained by using these tubes are significant. The bottles cost approximately 10% of the present price of Petri plates. Only half of the minimum amount of medium used in a Petri plate, and probably a third to a quarter of that generally used, is necessary. The time required for pouring medium into plates is eliminated by dispensing in the preparation room. The possibility of contamination is reduced. The required incubator space is reduced to a minimum, it being possible to accommodate 25 tubes in a 6 in. square basket or 100 on approximately 1 sq. ft. of shelf space.

Acknowledgments

The author wishes to acknowledge the information provided by Mr. A. Rowlands of the Institute for Research in Dairying, Shinfield, England, and the help and assistance of Mr. A. H. Northcott, instrument maker in Guelph, who built the spinner described.

References

1. FISHER, R. A., THORNTON, H. G., and MACKENZIE, W. A. Ann. Applied Biol. 9: 325-359. 1922.
2. HANNAY, C. L. Proc. Soc. Applied Bact. 2: 85-88. 1946.
3. JULIUS, H. W. Nederland. Tijdschr. Hyg. Microbiol. Serol. 5: 28-34. 1938.
4. ROWLANDS, A. Personal communication.
5. SNYDER, T. L. J. Bact. 54: 641-654. 1947.
6. WILSON, G. S. J. Bact. 7: 405-446. 1922.
7. WILSON, P. W. and KULLMAN, ETHEL. J. Bact. 22: 71-90. 1931.

ON THE FRICTION OF VARIOUS SYNTHETIC AND NATURAL RUBBERS ON ICE[1]

By P. M. Pfalzner

Abstract

The dynamic and static friction on ice, the hardness, and the resilience of natural and synthetic rubber tire compounds were measured. General relationships were found to exist between friction and hardness of the rubber, friction and ice temperature, hardness and temperature, hardness and age of the rubber, resilience and hardness. Extraction of the rubber had a variable effect.

Introduction

It is known that tires made of the standard GR–S tread compound are more apt to slip on icy roads than natural rubber tires; this has been reported both by the general public using such tires and confirmed by a number of investigators (1). It was felt to be desirable to obtain data on the frictional properties of rubber tire compounds on ice under controlled conditions with a view to discovering the factors which influence these properties. It was found that GR–S compounds can be prepared which are at least equal, as far as friction on ice is concerned, to comparable natural rubber compounds. Should these compounds prove to be satisfactory in other respects, they will contribute to safer driving and hence increase the confidence of the public in synthetic tires for winter use.

The Apparatus and Experimental Methods

The friction measurements were made in a cold-room which could be kept at constant temperatures between 32°F. and −10°F. On an electrically driven turntable a circular track was built (outside radius, 19 in.; width, 6 in.) into which water was poured and allowed to freeze. The surface, which usually froze unevenly in spite of being built up slowly, was smoothed by melting the top layer of ice with a cylindrical heating element which could be lowered to the track. Its axis was horizontal and at right angles to the direction of motion of the turntable. To prevent irregular refreezing, the water left on the melting surface was soaked up by a cotton cloth held just behind the heated cylinder.

The samples of rubber compounds were prepared by the Rubber Laboratory of the National Research Council under the direction of Mr. T. R. Griffith. Their dimensions were $5\frac{1}{2}$ by 1 by $\frac{1}{4}$ in.; they were cut into two equal lengths of $2\frac{3}{4}$ in., and were glued with rubber cement to pieces of soft wood of about the same size as the rubber pieces. The side to which the glue was applied

[1] *Manuscript received in original form March 24, 1949, and, as revised, August 8, 1949. Contribution from the Heat Section, Division of Physics, National Research Laboratories, Ottawa, Canada. Issued as N.R.C. No. 2260.*

was that which showed the imprint of markings from the mold in which the rubber compounds had been prepared, the other side being smooth. The pieces of wood bearing the rubber were then pressed against sharp metal tips protruding from the face of a block of wood $3\frac{1}{2}$ in. long and $2\frac{3}{4}$ in. wide, and held in place by these tips, the assembled arrangement having the appearance of a small sleigh with wide rubber runners. This method of attaching the rubber to the block permitted ready interchanging of the various rubber samples for test and yet gave a firm connection. The block of wood served as a loading platform for weights. A calibrated steel spring held the block and weights fixed while the turntable was in motion, and the extension of the spring measured the frictional force between the ice and rubber. When the dynamic friction was measured, the turntable was made to rotate at a uniform speed of about 50 r.p.m., and the load was increased from 0 to 6.7 lb. in steps of 1 lb. the last weight added being 0.7 lb., and then decreased to 0 again. Readings were taken of the force in ounces as indicated by the steel spring; they were averaged for each value of the load. When plotting force against load the points gave a straight line showing that the coefficient of friction is independent of the load within the range of loads used here. For comparison purposes it was thus decided to use only the force read at a load of 6.7 lb. Similarly when measuring static friction, the force read at a load of 3 lb. was used; the load was kept lower in this case since static friction is generally higher than dynamic and there was a limit to the amount the spring could be stretched; the turntable was moved slowly by hand until slipping occurred, and the average of several readings was taken.

The spring was attached at such a height that there was neither a tendency to lift nor to depress the block and the attached rubber samples.

The samples were placed in the cold-room upon being received from the Rubber Laboratory and tests were made as soon as possible after receipt. The dates of the various tests are indicated on the tables in order to show the length of time the samples had been exposed to cold conditions. The cold-room generally remained continuously below freezing except for some periods of mechanical breakdowns, but the temperature was sometimes 30°F., 0°F., −10°F., or intermediate for short periods. The usual operating temperature, however, was 0°F.

Hardness was measured with the Shore Instrument Company's Type A durometer; the readings, on a scale of 100, were taken 10 sec. after applying the durometer to the rubber surface.

Resilience was obtained with a resiliometer which measures the height to which a weight rebounds from the rubber; for this measurement the weight was dropped 10 times before taking a reading.

The ice surface represents a variable which enters into all the friction measurements. Although no pronounced trends attributable to "ice aging" were found, the figures are strictly comparable only for the same day, as the

surface may change owing to wear, condensation, or evaporation. Even on the same day, marked changes are quite possible; the surface may, for instance, become smoothed by the movement of the rubber runners, or, a contaminating film may be spread over it, either owing to settling dust or coming from the rubber itself, thus changing the friction. For this reason the results fluctuate but the general trends are nevertheless unmistakable.

The Apparent Area of Contact, Load, and Frictional Force

The law of friction $\mu = \frac{F}{W}$, where μ = coefficient of friction, F = frictional force, W = load, leads one to assume that the coefficient of friction between any two surfaces is a constant, independent of area of contact and load. The

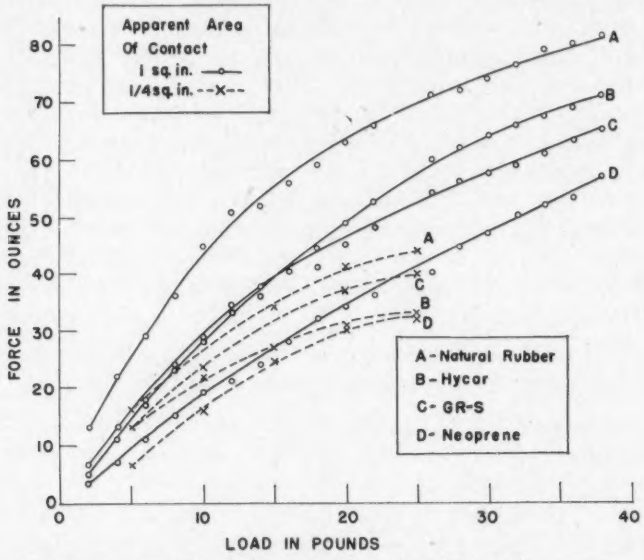

FIG. 1. *Relation between dynamic frictional force and load for natural rubber, GR–S, Hycar, and Neoprene compounds. Apparent areas of contact between ice and rubber 1 sq. in. and ¼ sq. in. Temperature, + 20°F.*

law is, however, only approximately true. The apparent area of contact between the macroscopically flat surfaces is generally many times the actual area of contact. Amontons' law of friction expresses the fact that over a certain range of loads the frictional force and load are proportional owing to the fact that the actual area of contact and load are proportional (2). Hence it is to be expected that if the load is increased to the point where the actual area of contact approaches a maximum, the coefficient of friction will fall off with further increases in load.

Experiments were made with natural rubber, Hycar, Neoprene, and GR–S at 20°F. on ice using apparent areas of contact of 1.0 sq. in. and 0.25 sq. in.

Loads up to 25 lb. (i.e., pressures up to 25 lb. per sq. in., and 100 lb. per sq. in., respectively) were used. The results (Table XV* and Fig. 1) show that the frictional force is initially proportional to the load, and falls off at high loads. For the same load, the smaller apparent area of contact has a lower friction; or, expressed another way, for the same pressure, the smaller apparent area of contact has a very much lower friction. However, within the range of loads used in the friction tests recorded in Tables I to XIV the frictional force and load were very nearly proportional, while the apparent areas were equal for all samples. The actual areas of contact will, of course, vary with the type of rubber; it is likely that the higher friction of the soft rubbers is largely due to the greater area of contact they have with the ice surface.

The Effect of Variations in Speed of Motion

The speed of motion of the turntable did not affect the dynamic coefficient of friction within the range used in these experiments (maximum, 8 m.p.h.), except at very low speeds when jerky motion occurred.

The Effect of Wet Ice Surface

Experiments showed that friction falls by as much as 50% on ice covered with a layer of water.

Experimental Results and Discussion

At the outset of the experiments there was little information at hand to guide the investigations. It is obvious that a wide number of chemical combinations is possible, and having no indication which factors are responsible for high frictional properties, a large number of combinations would have to be studied. However, it was thought that (*a*) hardness had undoubtedly a large influence on the friction, and (*b*) extraction, since it played a part in the abrasion resistance, might remove from the rubber certain ingredients affecting frictional properties. Hence, in the main, it was decided to investigate the influence of hardness and the effects of extraction on various selected rubber compounds, both natural and synthetic.

The Rubber Laboratory of the National Research Council have supplied the following details of the method of extraction used by them:

"Extraction was carried out in standard Soxhlet apparatus, without paper thimble. The solvent used was ethanol–toluene constant boiling mixture, made from 70 volumes of 95% ethanol and 30 volumes of toluene. The mixture of ethanol and toluene was purified before use by distillation; the liquid boiling at a temperature of approximately 75°C. was retained. The rubber was extracted for 96 hours with this ethanol–toluene azeotrope, followed by a 24-hour extraction with 95% ethanol to remove absorbed solvent from the rubber. The ethanol was changed four times during the 24-hour period. Half-way through the extraction with azeotrope the rubber strips

* *Table XV will be found on page 487.*

were turned end for end in the Soxhlet to ensure uniform extraction throughout the rubber."

In the tables of results, the samples tested in these experiments are divided into groups designated by capital letters. The groups usually consist of samples of a similar composition, with one ingredient varying in quantity from sample to sample. Each individual sample is designated by a number and its composition given in Tables XVI* to XXII.*

Tables I to XII give the frictional force in ounces measured for each sample with a load of 6.7 lb. for dynamic friction (and 3 lb. for static friction) at the temperature indicated; hardness is also given for each sample as measured with the durometer, greatest hardness being 100 on the scale of the instrument.

Tables I to III show the results for Samples A comprising numbers 5–8 to 5–15 unextracted and extracted. All of these are GR–S compounds. From Table I, considering at first only Samples 5–8 to 5–11, we note that the dynamic friction increases for all samples as the temperature is lowered. It will also be seen that although the temperature remained at 0°F. between August 14 and 15, the friction of the samples increased slightly; however, by December 17, still at 0°F., the friction has fallen considerably but is still higher than originally

TABLE I

DYNAMIC FRICTION: SAMPLES A

Sample No.	30°F. July 16	0°F. Aug. 14	0°F. Aug. 15	0°F. Aug. 21	0°F. Dec. 17
	Force in ounces				
(*a*) *Unextracted*					
5– 8	18	33	35		22
5– 9	17	33	35		23
5–10	16	31	33		23
5–11	15	30	38		23
5–12	12			25	14
5–13	12			37	9
5–14	20			58	26
5–15	21			52	14
(*b*) *Extracted*					
5– 8	16	37	46		27
5– 9	15	34	44		20
5–10	13	41	41		21
5–11	13	30	40		20
5–12	9			26	9
5–13	8			24	8
5–14	7			44	7
5–15	8			42	6

* *These tables will be found on pages 487 to 489.*

TABLE II

STATIC FRICTION: SAMPLES A

Sample No.	0°F. July 27	0°F. Aug. 17
	Force in ounces	
(*a*) *Unextracted*		
5- 8	30	24
5- 9	34	22
5-10	31	22
5-11	37	20
5-12	27	26
5-13	21	27
5-14	31	25
5-15	30	28
(*b*) *Extracted*		
5- 8		22
5- 9		22
5-10		25
5-11		21
5-12		20
5-13		19
5-14		20
5-15		22

TABLE III

HARDNESS OF SAMPLES A

Sample No.	70°F. Aug. 10	32°F. Sep. 24	0°F. Sep. 27	0°F. Dec. 16
(*a*) *Unextracted*				
5- 8	67	71	76	77
5- 9	68	68	76	78
5-10	65	67	75	78
5-11	67	70	75	79
5-12	63	73	82	82
5-13	53	64	83	80
5-14	65	66	70	73
5-15	52	59	67	73
(*b*) *Extracted*				
5- 8	57	67	75	75
5- 9	58	68	71	76
5-10	56	67	74	77
5-11	54	69	74	79
5-12	72	79	84	87
5-13	69	61	78	84
5-14	65	77	87	89
5-15	60	75	86	85

at 30°F. From Table II, we note that the static friction also falls with time even though the samples remain at the same temperature. Table III shows that the hardness increases with decreasing temperature, and the last two columns show a slow increase in hardness taking place between September and December at the same temperature. The rubbers are, however, very nearly equally hard, and their frictional properties cover only a narrow range.

Samples 5–12 to 5–15 give the same general results: increase in friction with lower temperature at first; then, after prolonged exposure to 0°F., a drop in friction which for 5–13 and 5–15 unextracted goes below the value first obtained at 30°F., and which takes 5–12, 5–13, and 5–14 extracted down to the same figures as obtained at 30°F., with 5–15 extracted going slightly below the

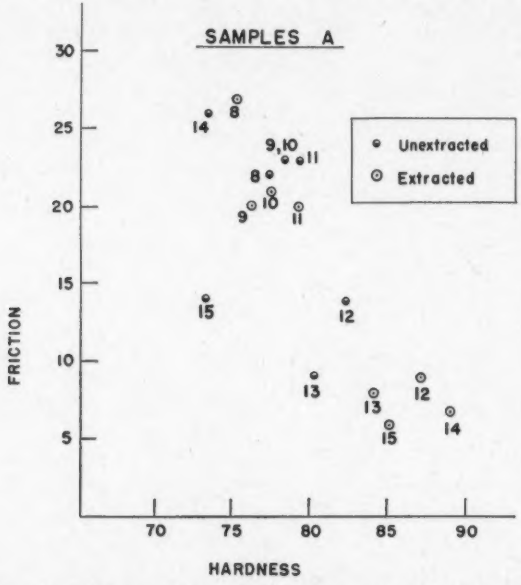

FIG. 2. *Relation between dynamic frictional force in ounces for a load of 6.7 lb. and hardness of rubber. Temperature, 0°F. Numbers refer to the sample number.*

original value; the hardness of these samples similarly increases with decreasing temperature and length of exposure to cold. The extracted rubbers 5–8 to 5–11 are at no time harder, while the extracted rubbers 5–12 to 5–15 are at no time softer, than the corresponding unextracted rubbers, when compared at the same temperature and for the same length of exposure to cold. Fig. 2 shows the relation between dynamic frictional force (in ounces at a load of 6.7 lb., at 0°F.) and hardness; the fall in friction with increasing hardness is clearly shown; the influence of extraction is less definite.

Tables IV to VI show results for samples C, comprising numbers 5–16 to 5–21, unextracted and extracted, all of which are GR–S compounds. The

TABLE IV

DYNAMIC FRICTION: SAMPLES C

Sample No.	0°F. Nov. 7	0°F. Dec. 17	30°F. July 16
	Force in ounces		
(a) *Unextracted*			
5–16	20	23	17
5–17	33	19	19
5–18	27	12	10
5–19	29	27	23
5–20	43	24	22
5–21	35	16	16
(b) *Extracted*			
5–16	33	20	17
5–17	33	26	21
5–18	18	20	15
5–19	43	21	19
5–20	38	31	24
5–21	28	24	21

TABLE V

STATIC FRICTION: SAMPLES C

Sample No.	0°F. Sep. 25	0°F. Nov. 8	30°F. July 18
	Force in ounces		
(a) *Unextracted*			
5–16	40	32	20
5–17	55	35	18
5–18	40	38	20
5–19	40	34	19
5–20	53	32	21
5–21	46	32	20
(b) *Extracted*			
5–16		33	20
5–17		35	23
5–18		32	17
5–19		36	18
5–20		39	20
5–21		33	22

TABLE VI

HARDNESS OF SAMPLES C

Sample No.	70°F. Sep. 17	32°F. Sep. 24	0°F. Sep. 27	0°F. Dec. 16
(*a*) *Unextracted*				
5–16	65	63	70	73
5–17	49	62	70	72
5–18	61	72	82	85
5–19	60	60	64	67
5–20	46	54	62	65
5–21	55	62	74	75
(*b*) *Extracted*				
5–16	61	63	71	75
5–17	53	47	52	55
5–18	66	65	69	72
5–19	55	61	66	70
5–20	47	47	51	54
5–21	54	53	60	60

conclusions drawn from these tables are very similar to those obtained with Samples A. The dynamic and static friction increase with decreasing temperature. The friction decreases with prolonged exposure to cold as the rubber gradually hardens. At room temperature, the extracted rubbers do not differ appreciably in hardness from the unextracted ones; after extraction,

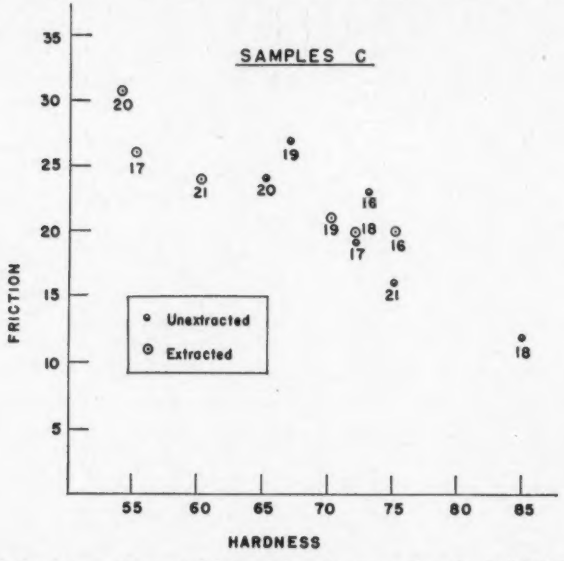

FIG. 3. *Relation between dynamic frictional force in ounces for a load of 6.7 lb. and hardness of rubber. Temperature, 0°F. Numbers refer to the sample number.*

three samples, 5–16, 5–19, and 5–21 are softer, and the remaining three are harder than originally. At 32°F., however, only one of the extracted rubbers, 5–19, is harder than the unextracted; all the others are softer except for 5–16, which is unchanged. At 0°F. on September 27, all the extracted rubbers except 5–16 and 5–19 are softer, and on December 16 at 0°F. the same is true, all the rubbers having hardened in the interval. For Samples C, then, it will be noted that the unextracted rubbers harden more, in general, with decreasing temperature and length of exposure than the extracted rubbers. A general relation between hardness and dynamic friction is apparent from Fig. 3: on December 17, at 0°F., 5–18 unextracted has the lowest dynamic friction and is also the hardest of the rubbers, while 5–19 and 5–20 have highest friction and are the two softest rubbers. Among the extracted rubbers, 5–17 and 5–20 are softest and have the highest dynamic friction; 5–16 and 5–18 are hardest and have the lowest friction. This relation does not extend to static frictional properties; 5–18 unextracted is hardest, while 5–20 extracted is softest and both have highest static friction at 0°F. At 30°F. the frictional properties of the rubbers have a narrower range of values. Extraction has a variable effect, sometimes softening the rubber and increasing dynamic friction, sometimes producing the opposite result. An extracted rubber may also initially become softer and subsequently harden in excess of the unextracted rubber, e.g., 5–16 which has hardness 65 and 61 at 70°F. for unextracted and extracted respectively, and 73 and 75 at 0°F. on December 16.

In order to obtain a clearer picture of the influence of hardness on natural and synthetic rubbers, Samples J were made up. Numbers 5–52 to 5–55 are GR–S synthetic compounds, graded in hardness from about 45 to 88 at room temperature, and numbers 5–56 to 5–59 are corresponding natural rubber compounds whose hardness is as closely as possible equal to that of the synthetic compounds. The increase in friction with decreasing hardness is shown in Fig. 4. From Table VII it will be seen that there is little difference in the dynamic friction of the synthetic and natural rubbers, and that synthetic

TABLE VII

DYNAMIC FRICTION: SAMPLES J

Sample No.	30°F. Sep. 15	30°F. Sep. 25	0°F. Oct. 4
	Force in ounces		
5–52	24	28	52
5–53	15	18	27
5–54	11	11	17
5–55	8	7	12
5–56	26	31	50
5–57	12	19	31
5–58	8	12	17
5–59	7	8	12

TABLE VIII

STATIC FRICTION: SAMPLES J

Sample No.	28°F. Sep. 16	30°F. Sep. 24
	Force in ounces	
5–52	29	26
5–53	26	22
5–54	21	14
5–55	15	11
5–56	31	25
5–57	28	25
5–58	26	19
5–59	22	14

TABLE IX

HARDNESS OF SAMPLES J

Sample No.	70°F. Sep. 4	30°F. Sep. 24	0°F. Sep. 27	0°F. Oct. 4
5–52	45	45	52	52
5–53	60	62	70	69
5–54	74	78	87	86
5–55	88	91	96	96
5–56	44	43	49	48
5–57	63	65	72	73
5–58	78	82	87	89
5–59	88	92	95	95

TABLE X

RESILIENCE: SAMPLES J

Sample No.	0°F. June 5
5–52	10
5–53	10
5–54	13
5–55	19
5–56	15
5–57	17
5–58	20
5–59	20

compound 5–52 has excellent frictional properties, being only slightly inferior to natural compound 5–56 at 30°F., and superior at 0°F. When it is considered that 5–52 is somewhat harder than 5–56, it will be realized that this compound is at least the equal of the particular natural rubber compound 5–56.

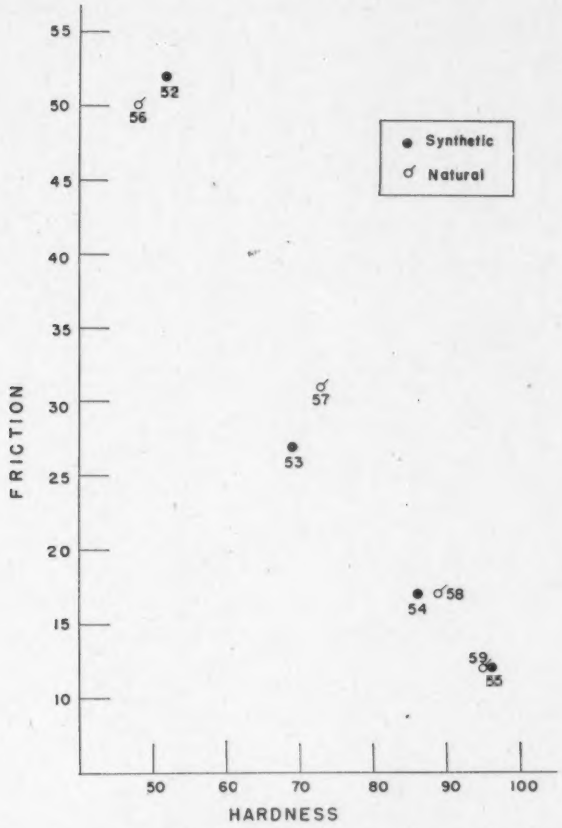

FIG. 4. *Relation between dynamic frictional force in ounces for a load of 6.7 lb. and hardness of rubber. Temperature, 0°F. Numbers refer to the sample number.*

Tables XI to XIII show results for Samples M, which, like Samples J, fall into two groups of graded hardness, and, in addition, have also been extracted. Numbers 35–5 to 35–8 are natural rubber compounds, increasing in hardness from 37 to 84 at room temperature, and numbers 35–9 to 35–12 are GR–S synthetic compounds of corresponding hardness. Considering first the hardness of the rubbers (Table XIII) a difference will be noted in the behavior of extracted natural and synthetic compounds. The hardness of all these

TABLE XI

DYNAMIC FRICTION: SAMPLES M

Sample No.	0°F. Jan. 27	0°F. June 17
	Force in ounces	
(*a*) *Unextracted*		
35–5	68	58
35–6	36	24
35–7	19	15
35–8	17	9
35– 9	60	48
35–10	36	28
35–11	15	14
35–12	12	10
(*b*) *Extracted*		
35–5	59	18
35–6	27	10
35–7	18	6
35–8	13	4
35– 9	38	33
35–10	21	19
35–11	15	10
35–12	9	6

TABLE XII

STATIC FRICTION: SAMPLES M

Sample No.	0°F. June 17
	Force in ounces
(*a*) *Unextracted*	
35–5	40
35–6	34
35–7	37
35–8	33
35– 9	40
35–10	38
35–11	34
35–12	35
(*b*) *Extracted*	
35–5	28
35–6	25
35–7	25
35–8	25
35– 9	34
35–10	30
35–11	30
35–12	27

TABLE XIII

HARDNESS OF SAMPLES M

Sample No.	70°F. Jan. 2	0°F. Jan. 27	0°F. June 17
(*a*) *Unextracted*			
35–5	37	42	46
35–6	52	62	79
35–7	72	80	90
35–8	84	89	97
35– 9	36	42	45
35–10	50	62	60
35–11	67	80	78
35–12	78	92	93
(*b*) *Extracted*			
35–5	35	45	81
35–6	54	68	95
35–7	70	86	99
35–8	84	95	100
35– 9	37	52	50
35–10	49	75	68
35–11	61	92	87
35–12	77	98	95

TABLE XIV

RESILIENCE: SAMPLES M

Sample No.	0°F. June 5
(*a*) *Unextracted*	
35–5	21
35–6	14
35–7	18
35–8	26
35– 9	11
35–10	12
35–11	10
35–12	20
(*b*) *Extracted*	
35–5	12
35–6	17
35–7	24
35–8	19
35– 9	11
35–10	13
35–11	20
35–12	22

samples increases with decreasing temperature; the unextracted natural rubbers 35–5 to 35–8 all harden by small amounts between January and June at 0°F., while the extracted natural rubbers harden considerably in the same period. This should reduce the friction of these extracted compounds, and Table XI shows that this happens. The extracted natural rubbers are much harder and have poor friction. Considering now the synthetic compounds 35–9 to 35–12, it will be noted from Table XIII that the unextracted rubbers change little in hardness during the period of exposure to 0°F. from January to June; in fact samples 35–10 and 35–11 become softer; thus, by June, these

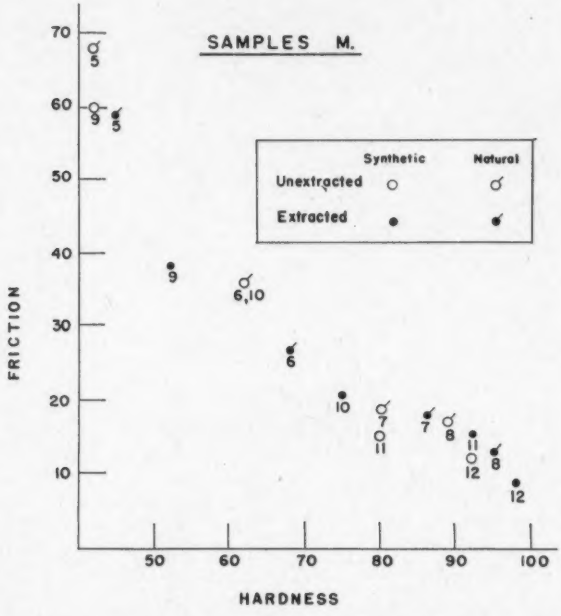

FIG. 5. *Relation between dynamic frictional force in ounces for a load of 6.7 lb. and hardness of rubber. Temperature, 0° F. Numbers refer to the sample number.*

rubbers are all softer than the corresponding natural compounds. The extracted synthetic compounds 35–9 to 35–12 harden at first more between 70°F. and 0°F. than the unextracted but they all become softer again with prolonged exposure to 0°F. By June, they still are slightly harder than the unextracted synthetics, but softer than the unextracted natural compounds (except for 35–9 which is harder than 35–5) and much softer than the extracted natural rubbers. Hence, if hardness alone were the determining factor, we would expect the synthetic unextracted compounds 35–9 to 35–12 to be slightly superior both to the corresponding extracted samples 35–9 to 35–12 and the

unextracted natural compounds 35–5 to 35–8, and much superior to the extracted natural compounds 35–5 to 35–8. Table XI and Fig. 5 show that the first and third expectations are borne out, while the second holds only for 35–10 and 35–12. Thus hardness is not decisive in every case.

Table XII shows that the static friction does not vary as greatly with hardness as the dynamic friction. The unextracted compounds are slightly better than the extracted ones, and the synthetics are better than the natural rubbers.

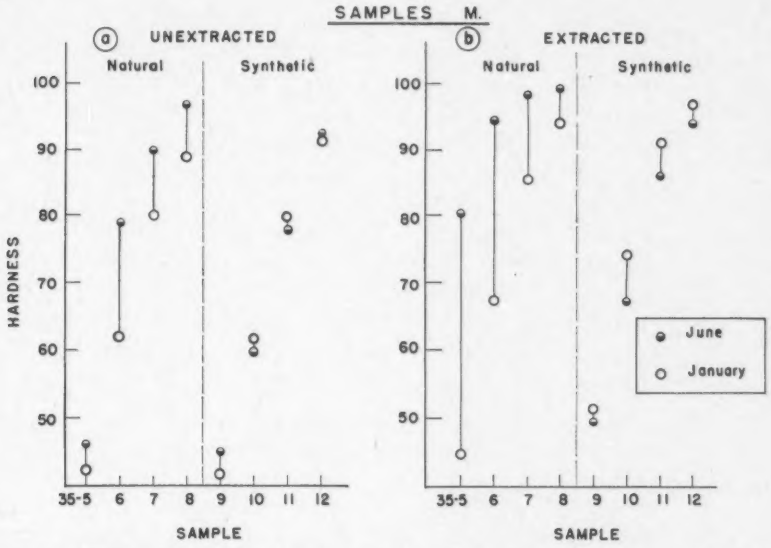

FIG. 6. *Hardness of rubber samples M. Variation with age of rubber. Temperature, 0°F.*

The resilience of Samples J and M is shown in Tables X and XIV. It will be noted that the resilience increases in general with hardness, but that exceptions are common. Each of the rubbers 35–9 to 35–12 unextracted has lower resilience than corresponding rubbers in the other groups, except 35–8 extracted; thus low resilience appears to be favorable to high friction.

Fig. 6 shows the change in hardness with age of Samples M. It will be noted that the natural rubbers harden considerably on prolonged exposure to cold, whereas the synthetics become slightly softer.

The change in hardness with temperature for all the rubbers is shown in Figs. 7 and 8. The hardening at lower temperatures occurs in every case between 30°F. and 0°F., but not always between 70°F. and 30°F.

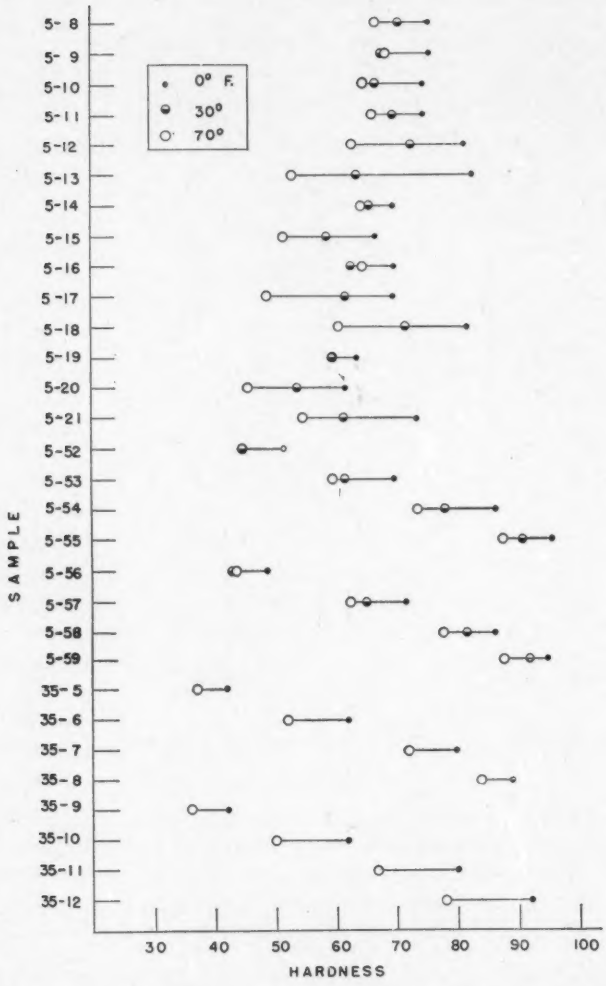

FIG. 7. *Hardness of the unextracted rubber samples. Variation with temperature.*

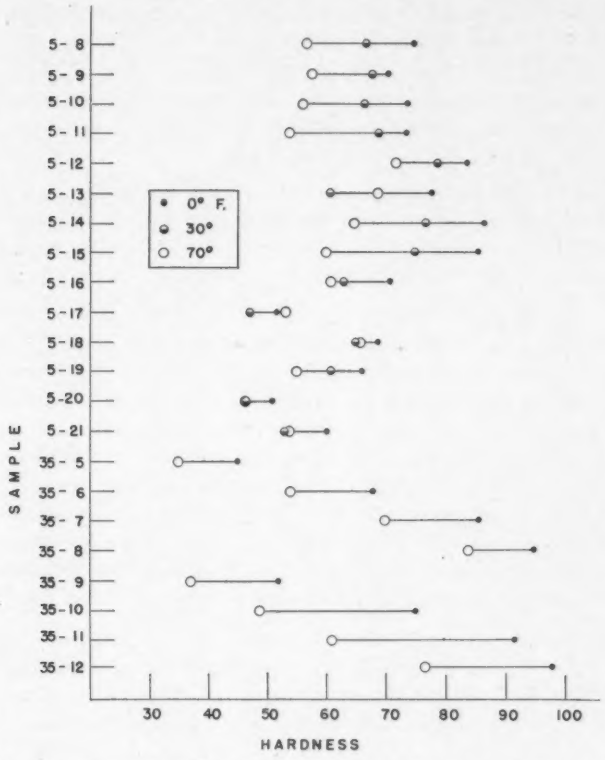

FIG. 8. *Hardness of the extracted rubber samples. Variation with temperature.*

General Conclusions

Taking all the information presented in the tables and figures together, the following general observations may be made:

1. The hardness increases at lower temperatures (with the exception of a few compounds). (Figs. 7 and 8)

2. The friction increases at lower temperatures. In a few cases, however, the rubber hardens excessively at the lower temperature and the friction is thereby reduced. (Tables I to XIV)

3. A harder rubber has a lower friction than a softer rubber of the same general type. (Figs. 2 to 5)

4. The extracted rubbers are generally softer immediately after extraction than the unextracted, and have a higher dynamic friction. (Figs. 2 and 3, 4, 5, 7, 8)

5. The natural rubber compounds become harder on prolonged exposure to cold, the extracted natural rubbers showing a larger increase in hardness than

the unextracted; the synthetic rubber compounds, extracted and unextracted, change little or become softer. (Fig. 6)

6. The dynamic friction of the extracted natural rubber compounds, after prolonged exposure to cold, falls below that of the unextracted, while the extracted synthetic compounds usually have a higher friction than the unextracted. (Fig. 6, Tables VII, IX, XI, XIII)

7. Static and dynamic friction are not equally affected by extraction; the extracted rubber may have a higher dynamic friction but a lower static than the unextracted, or vice versa. The influence of hardness on static friction is not comparable to its pronounced effect on dynamic friction. (Tables I to XIII)

There still remains the question of whether it is the dynamic friction or the static friction which decides the running performance of rubber tires on a slippery road. It is clear that, when starting a motor vehicle, a high static friction is desirable to prevent spinning of the wheels, or when stopped on an incline, to prevent sliding downhill; a high dynamic friction is desirable when the wheels are locked and sliding, to bring the car to a stop; or when spinning the wheels, to set the car in motion. When the car is in motion with the wheels turning, however, it is not possible to say what type of friction will prevent the occurrence of sliding or slipping. It is therefore not to be expected that the laboratory results will in every case be borne out by actual road tests.

Attention must be drawn to the fact that no attempt has been made in this report to interpret the results in terms of the chemical composition of the samples. Probably many more tests will be required before the rubber chemists have sufficient data to understand the effect of composition on frictional properties.

Acknowledgments

The author's thanks are due to Dr. C. D. Niven for constant encouragement and valuable discussions, to the Rubber Laboratory of the Division of Chemistry, National Research Council, for the preparation of the samples, and in particular to Mr. T. R. Griffith, head of that laboratory, for guiding the composition of the samples.

References

1. CONANT, F. S., DUM, J. L., and COX, C. M. Ind. Eng. Chem. 41: 120-126. 1949.
2. SCHNURMANN, R. J. Applied Phys. 11: 624. 1940.

TABLE XV

SHOWING DYNAMIC FRICTIONAL FORCE IN OUNCES FOR LOADS VARYING FROM 0 TO 42 LB. WITH FOUR TYPES OF RUBBERS, SURFACE AREAS 1 SQ. IN. AND ¼ SQ. IN. (SEE ALSO FIG. 1)

Load, lb.	Natural rubber		Neoprene		Hycar		GR-S	
	1 sq. in.	¼ sq. in.	1 sq. in.	¼ sq. in.	1 sq. in.	¼ sq. in.	1 sq. in.	¼ sq. in.
	Ounces		Ounces		Ounces		Ounces	
0	0	0	0	0	0	0	0	0
2	13	—	3.5	—	5	—	7	—
4	22	—	7	—	11	—	13	—
5	—	16	—	6.5	—	13	—	16
6	29	—	11	—	17	—	18	—
8	36	—	15	—	23	—	24	—
10	45	23	19	16	28	22	28	23.5
12	51	—	21	—	34.5	—	33	—
14	52	—	24	—	37.5	—	36	—
15	—	34	—	24.5	—	27	—	34
16	56	—	28	—	40	—	40	—
18	59	—	32	—	44.5	—	41	—
20	63	41	34	30	49	30.5	45	37
22	66	—	36	—	52.5	—	48	—
25	—	44	—	32	—	33	—	40
26	71		40		60		54	
28	72		44.5		62		56	
30	74		47		64		57.5	
32	76.5		50		66		59	
34	78		52		67.5		61	
36	80		53		69		63	
38	82		57		71		65	
42	85		60		76		70	

The following tables give the formulas of the samples.

TABLE XVI

EFFECT OF VARIATION OF SOFTENER CONTENT

Sample No.	5–8	5–9	5–10	5–11
GR-S	100	100	100	100
Wyex	50	50	50	50
Zinc oxide	5	5	5	5
B.R.T. No. 7	—	2.5	5	10
Captax	1.5	1.5	1.5	1.5
Sulphur	2	2	2	2
Cure at 292°F. min.	90	90	90	90

TABLE XVII
NONBLACK COMPOUNDS

Sample No.	5–12	5–13	5–14	5–15
GR–S	100	100	100	100
Paraffin	1	1	1	1
Calcene T	202	202	—	—
Silene EF	—	—	64.5	64.5
Agerite powder	2	2	2	2
Zinc oxide	5	5	5	5
Sulphur	2.5	2.5	2.5	2.5
Cumate	0.75	0.75	0.75	0.75
Cumar MH2½	—	15	—	15
Cure at 316°F. min.	15	15	15	15

TABLE XVIII

Sample No.	5–16	5–17	5–18	5–19	5–20	5–21
GR–S	100	100	100	100	100	100
Wyex	50	50	70	35	35	45
Catalpo Clay	—	—	—	30	30	50
3–GP–26, mineral oil	—	20	20	—	20	20
B.R.T. No. 7	5	5	5	5	5	5
Zinc oxide	5	5	5	5	5	5
Sulphur	2	2	2	2	2	2
Captax	1.5	1.5	1.5	1.5	1.5	1.5
Cure at 292°F., min.	60	60	60	60	60	60

TABLE XIX
EFFECT OF HARDNESS, GR–S COMPOUNDS

Sample No.	5–52	5–53	5–54	5–55
GR–S	100	100	100	100
Wyex	25	50	75	100
Bardol	6	6	6	6
Pine tar	4	4	4	4
Sulphur	2	2	2	2
Zenite A	1.7	1.7	1.7	1.7
Stearic acid	1	1	1	1
Santoflex BX	1	1	1	1
Zinc oxide	5	5	5	5
Cure at 310°F., min.	30	30	30	30

TABLE XX
EFFECT OF HARDNESS, NATURAL COMPOUNDS

Sample No.	5–56	5–57	5–58	5–59
Smoked sheet	100	100	100	100
Reogen	2	2	2	2
Stearic acid	3	3	3	3
Pine tar	1	1	1	1
Agerite powder	2	2	2	2
Zinc oxide	3	3	3	3
Wyex	25	50	75	100
Sulphur	3	3	3	3
Captax	1	1	1	1
Cure at 292°F., min.	25	25	25	25

TABLE XXI

Sample No.	35–5	35–6	35–7	35–8
Smoked sheet	100	100	100	100
Reogen	2	2	2	2
Stearic acid	3	3	3	3
Pine tar	1	1	1	1
Circo light process oil	10	10	10	10
Agerite powder	2	2	2	2
Zinc oxide	3	3	3	3
Wyex	25	50	75	100
Sulphur	3	3	3	3
Captax	1	1	1	1
Cure at 292°F., min.	25	25	25	25

TABLE XXII

Sample No.	35–9	35–10	35–11	35–12
GR–S, Sarnia	100	100	100	100
Wyex	25	50	75	100
Bardol	6	6	6	6
Circo light process oil	10	10	10	10
Pine tar	4	4	4	4
Zenite A	1.7	1.7	1.7	1.7
Stearic acid	1	1	1	1
Santoflex BX	1	1	1	1
Zinc oxide	5	5	5	5
Sulphur	2	2	2	2
Cure at 310°F., min.	30	30	30	30

THE MEASUREMENTS OF STRAINS IN TIRES[1]

By D. L. Loughborough, J. M. Davies,[2] and G. E. Monfore[3]

Abstract

Because of obvious complications it is too difficult to calculate the stresses in the cords of a tire. In this work the direct measurement of strains was undertaken. Two techniques will be discussed; (1) the measure of the increase in length of the cords in the outside ply, and (2) a radiographic technique in which the change in length of the cord elements, marked by steel wires, is followed by photographic methods. The measurements on a number of tires are presented. The cords in a tire are strained about 2% by the inflation pressure. When the tire is loaded some of the cords are thrown into compression and some into more tension. During the rotation of the tire the cords are thus subjected to a vigorous alternation of compression and tension (± 2%). At the same time the angles between the cords change by as much as two degrees. A few general applications are mentioned and their importance to the tire engineer is indicated.

Introduction

The load on a tire is carried mainly by the cords in the carcass structure. The magnitude of the strains is important because the performance of the tire, especially the resistance to failure by cord fatigue, bruise breaks in the cords, or ply separation, depends directly on these strains. Since the tire structure is too complicated to allow the strains to be calculated, the experimental approach was taken. Preliminary tests were made on a number of tires and then strains on a single tire were determined in considerable detail.

Observation of a Tire at High Deflection

The wrinkle which develops in the low sidewall of an underinflated tire has been seen by most car users. Actually this wrinkled condition develops very gradually as the tire is deflected and though it can not be seen in a 7: 60–15 tire until the deflection has reached about 2.5 in., it is easily detected at much lower deflections by the ease with which the sidewall can be deformed laterally. This arises because the bending of the sidewall in two directions produces a shear with a resulting force opposed to the pressure. When the two forces are equal, a buckle occurs. Even in normal service such forces are present and contribute largely to some kinds of tire failure. Such observation led to the studies reported here.

Preliminary Measurement

The state of strain in the cords in the outside ply of tires can be rapidly assessed by examination of a tire after the sidewall rubber has been removed. A small circle (1-in. diameter) (located by the distance from the center of the

[1] *Manuscript received July 31, 1950.*
Contribution from the B. F. Goodrich Company Research Center, Brecksville, Ohio.
[2] *Now employed by the Army Quartermasters Corps, Textile Research & Development Laboratory, Philadelphia, Pennsylvania.*
[3] *Now employed by the Bureau of Reclamation, Denver Federal Center, Denver, Colorado.*

circle to the rim line and by the angle the line connecting its center with the center of rotation makes with the load axis) will be deformed when the tire is loaded. The strain in the cord can be determined by measuring the new cord length.* Compressions are observed on that side of the tire in which the cords run from the bead into the contact area. On the opposite side of the load axis and the same side of the tire the stresses are tension stresses. The compression increases rapidly from 0 to 20° from the load axis and falls off more slowly thereafter. The effect does not disappear at angles as high as 85°. The compression decreases from rim to shoulder, though with the technique being described it is not possible to examine the tire very far up the sidewall. Measurements are more easily made at high deflection where strains are high but without affecting the functional dependence on cord angle, observation angle, distance from rim, etc. When the load is increased enough to produce a maximum cord compression of about 15%, the tire wrinkles.

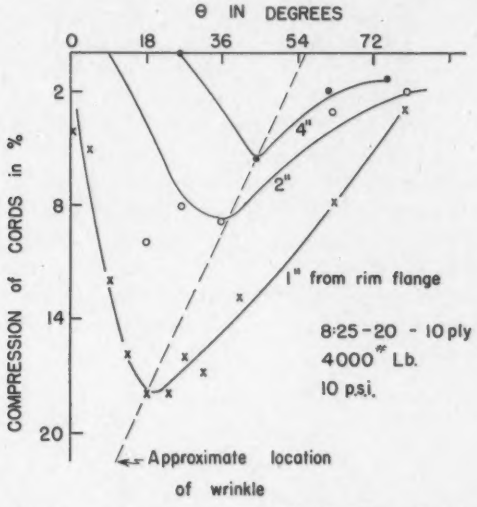

FIG. 1. *Cord compression measured on the outside ply as a function of position in the tire.*

In Fig. 1 some measurements on an 8: 25–20 truck tire under 4000 lb. load and 10 p.s.i. inflation pressure are presented. This extreme deflection was chosen to exaggerate the condition. The values for more nearly normal conditions are shown in Fig. 2. In general it has been found that the state of strain in the cords is largely determined by the tire deflection.

The position at which the maximum compression is produced depends primarily on the cord angles in the tire. For example, a tire with a 30° crown angle had the position of maximum compression 27° from the load axis while that for a tire with a 38° crown angle came at 21°.

* *The cord used in these tires had a modulus of about 1 lb. per per cent elongation. Until evidence is secured it is unwise to use this figure to calculate stresses.*

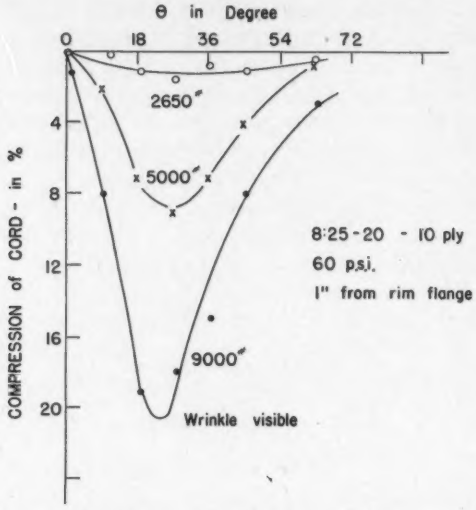

FIG. 2. *Cord compression on the outside ply as a function of tire deflection.*

In the figures the compression has been referred to the uninflated tire. Actually the cords are elongated slightly by tire inflation. This can also be assessed with rather poor accuracy, by a technique similar to that just described by computing the average value around the tire. For four tires (constructed of the same cord) the results are given in Table I as they depend on inflation pressure.

TABLE I

CORD STRAIN AS A FUNCTION OF INFLATION PRESSURE

	– At no load –			
Tire size	7:00–20	8:25–20	10:00–20	11:00–20
No. of plies	8	10	12	12
Cords/in.	190	240	290	290
Gauge pressure, p.s.i.	Strain, %			
0	0	0	0	0
40	2.5	1.7	1.0	0.9
80	3.1	2.4	1.7	2.2
120	4.0	3.3	2.6	2.8

The cord compression may be produced by a lateral thrust, such as that to which the tire is subjected during cornering, as well as by a radial load. A 6:00–16 4 ply tire inflated to 32 lb. and loaded to 2200 lb. was subjected to a force normal to the plane of the tire and applied at the contact area. The cord compression was measured and the radial load necessary to produce this same compression was determined. It should be realized that the tire is not

completely symmetrical, for a side load increases compression on one side of a tire and decreases it on the other (recorded here as thrust and pull). Table II shows that a lateral thrust contributes very materially to the strain in the cords of a tire.

TABLE II

EFFECT OF LATERAL THRUST ON CORD COMPRESSION 2200 LB. RADIAL LOAD

Lateral force		Max. cord compression, %	Radial load to give same compression, lb.
500 lb.	Thrust	1.4	1000
300		3.0	
0		9.2	2200
200		12.8	
300	Pull	13.4	2800
500		15.8	

Constructional features affect the extent and type of stress distribution considerably. A few of these features can be listed.

(1) The type of cord from which the tire is built determines to some extent the compression in the cords. Tires made with low twist cords show wrinkles at earlier loads.
(2) The effect of cord angle has just been considered.
(3) A feature which has a real effect on the state of strain in a tire is the width of the rim on which the tire is mounted. Presumably this effect arises because the sidewalls are made more vertical and hence less subject to double bending required by the deformation.
(4) Cord count, number of plies, amount of rubber between plies and similar features make minor contributions in changing the cord strain.

TABLE III

EFFECT OF RIM WIDTH

8: 25–20 60 p.s.i. 11,000 lb. load

Rim width, in.	Max. compression,
3.75	17.0
5.00	14.8
6.00	14.8
7.33	14.2
8.37	11.5

6:00–16 4 ply tire 30 p.s.i.

Load	Max. compression, %	
	3.62 F rim	4.50 K rim
0	−3.0%	−3.0%
900	.4	1.0
1350	3.6	4.0
2000	7.0	8.0
2500	10.8	12.0
3000	Tire wrinkled	15.0

The technique described measures only the stress in the outside ply of a tire and only in a limited region. This method is easy and should have considerable value for a tire engineer who wishes to evaluate constructional changes. It leaves much to be desired for the experimenter who requires a knowledge of the stress throughout the tire. This can be achieved by a tedious method involving a radiographic technique. Data for one tire will be given in detail.

Measurement of all Plies Radiographically

The tire studied was a 6 ply 6:00–16 with dipped cotton cords in an all synthetic build-up. The maximum rated load was 1065 lb. at an inflation pressure of 36 p.s.i. The tire was mounted on a 4.50 E rim and under the standard conditions the deflection was 12% of the section height.

The method of measurement involved placing markers in the tire as it was built. These markers were fine steel wires, 10 mils in diameter, wound tightly around the cords. Two markers were placed about one-half inch apart and differences in the distances between these markers gave the change in length of the cord. For increased accuracy short lengths of the wire, about one-half inch long, were placed beside the cords. All changes in distance between markers were determined by taking X-ray pictures. Such markers were placed in each ply, near the middle of the sidewall, at the shoulder and at the crown. Fig. 3 is an X-ray picture showing these markers as built into the tire on the flat drum. The picture was taken before forming. In this picture the

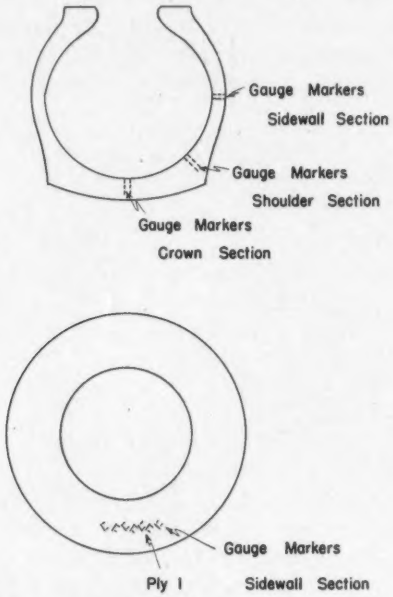

FIG. 4. *Location of gauge markers in cured tire.*

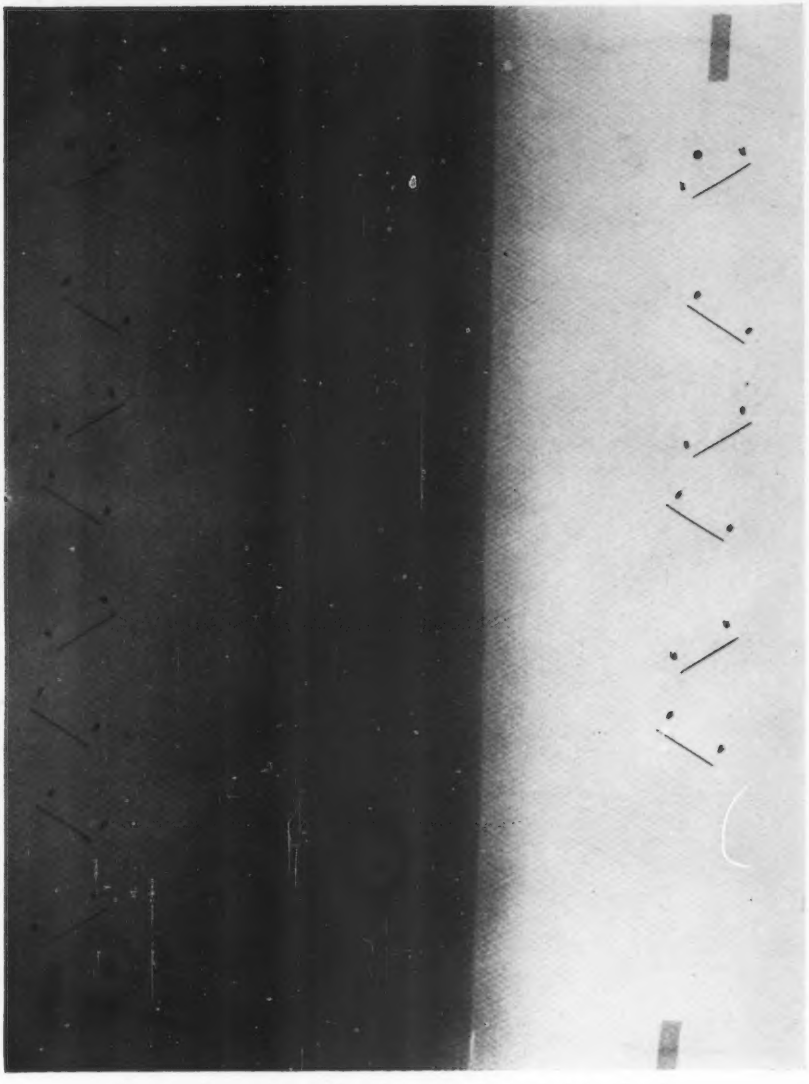

FIG. 3. *X-ray photograph of the markers in the tire before forming.*

length markers and the straight length of wire can be clearly seen. For measurement purposes these were enlarged 10 times optically. The location of the markers is further shown in Fig. 4.

The strains in the cords were determined for various inflation pressures and various loadings. For these measurements the X-ray film was placed inside the tire between the tire and the tube. The tire, mounted on the rim, was placed in a loading frame and deflected the desired amounts by adjusting the parallel loading plates. The X-ray unit was a General Electric Type KX-10 operated at 60 kv. It was about 8 ft. from the tire and exposures of from 5 to 10 min. were required. The loading frame ready for exposure is shown in Fig. 5.

FIG. 5. *The tire, loading frame, and X-ray camera.*

Results

On inflating the tire apparently all of the cords stretch slightly. Typical results are shown in Fig. 6, and for the normal inflation pressure the elongation is about 2%. This is measured relative to the length with the tire uninflated.

The behavior under load is considerably more complicated (Fig. 7). In some locations the cords are stretched and in others compressed. Typical results include the effect of inflation pressure and load, i.e., its length was compared with the corresponding length in the uninflated tire. In the first

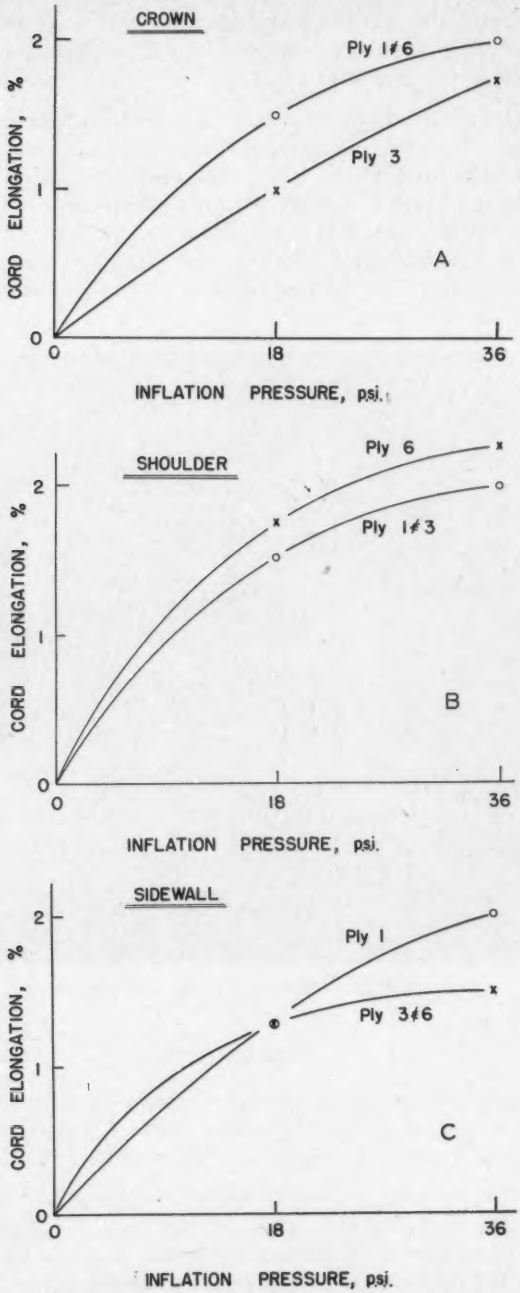

FIG. 6–A, B, C. *Effect of tire inflation pressure on cord strain.*

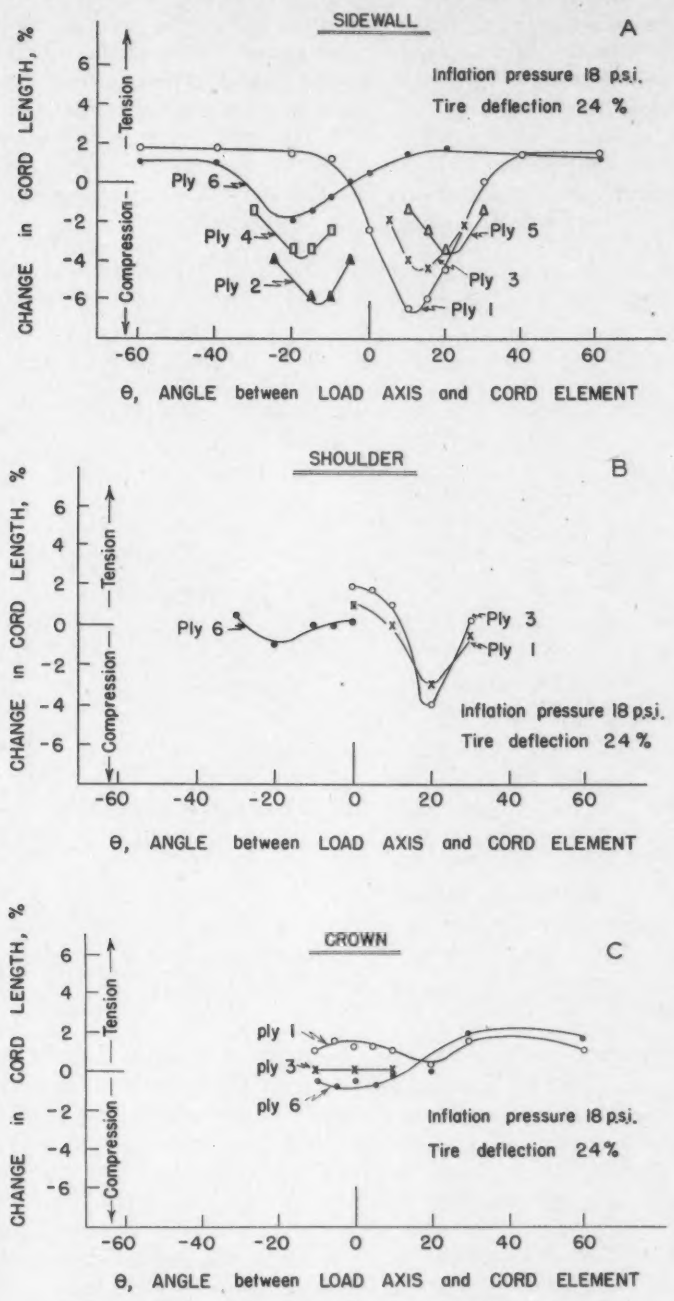

FIG. 7–A, B, C. *Compression in the cords produced by tire load.*

place, the maximum compression does not occur on the loading axis but at 12 to 20° from the axis. As might be expected, the maximum compression occurs in the first ply. The results here are exaggerated because the deflection was double the normal value, but for these conditions the maximum compression amounted to more than 6%. The compression in the sidewall is considerably greater than at the shoulder. At the crown in general the cords are stretched. As is expected from the direction of the cords the compression exists only in alternate plies on each side of the loading axis.

The results shown in Fig. 8 indicate a very large dependence of the amount of compression on deflection, but even under normal conditions the cords are

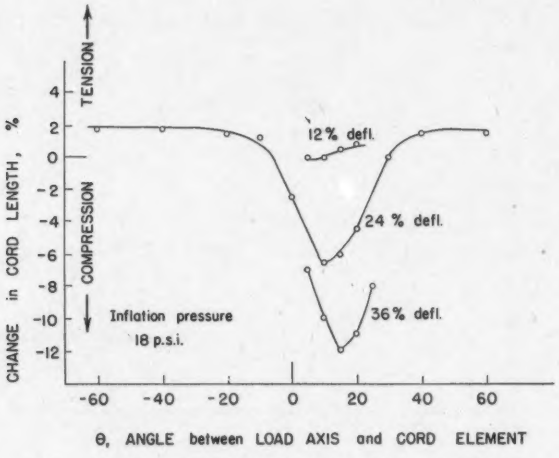

FIG. 8. *The effect of tire deflection on the cord strains.*

under compression over part of their length during a tire revolution. The results in Fig. 9 show that even at the same deflection there is some dependence on inflation pressure and the compression is greater at the lower inflation pressure.

Some indication of the relative motion of adjacent plies is given in the next two figures. Since the markers and adjacent plies were not directly above one another there is some inaccuracy here. Furthermore, these results show only relative rotation of the cords and there may be in addition some translational motion. In all cases the result is expressed as the change in angle between cords in adjacent plies and shows the relative twisting due to inflation or load. The angles in the uninflated tire were 74° at the crown, 78° at the shoulder, and 90° in the sidewall.

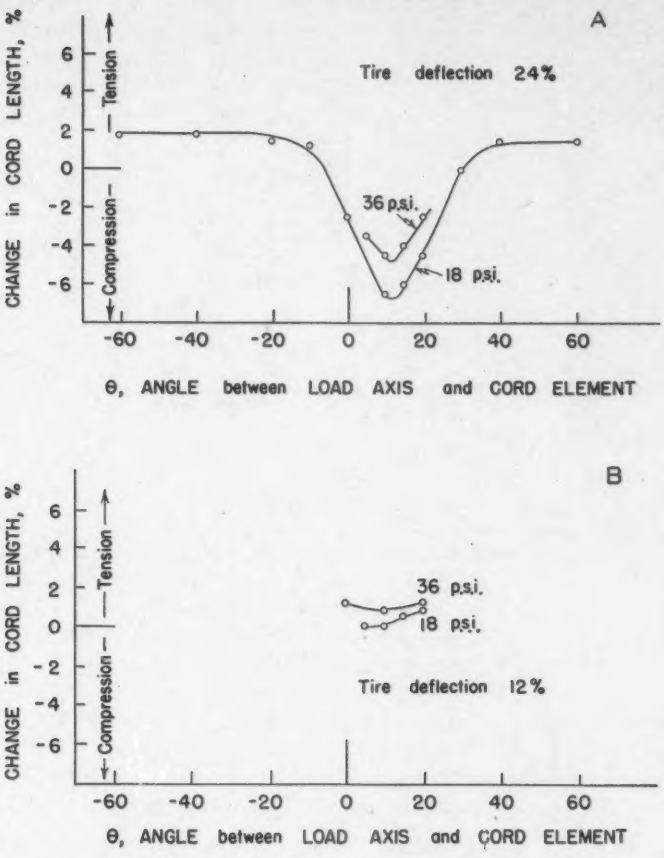

FIG. 9–A, B. *The effect of inflation pressure on cord strains.*

Fig. 10 shows the motion resulting from inflation pressure. For most cases it is in the neighborhood of 1° but in the sidewall it can amount to as much as 5°.

The effect of load on the relative motion of the cords is shown in Fig. 11.

The figures include the effect of inflation and load, i.e., the angles are compared to those in the uninflated tire. Here again the maximum change in angle occurs in the region around 20° from the loading axis. The relative cord angles change by as much as 7° and there is greater change in the sidewall and crown than at the shoulder. Again these results were obtained at an exaggerated tire deflection and doubtless the change in cord angle would be

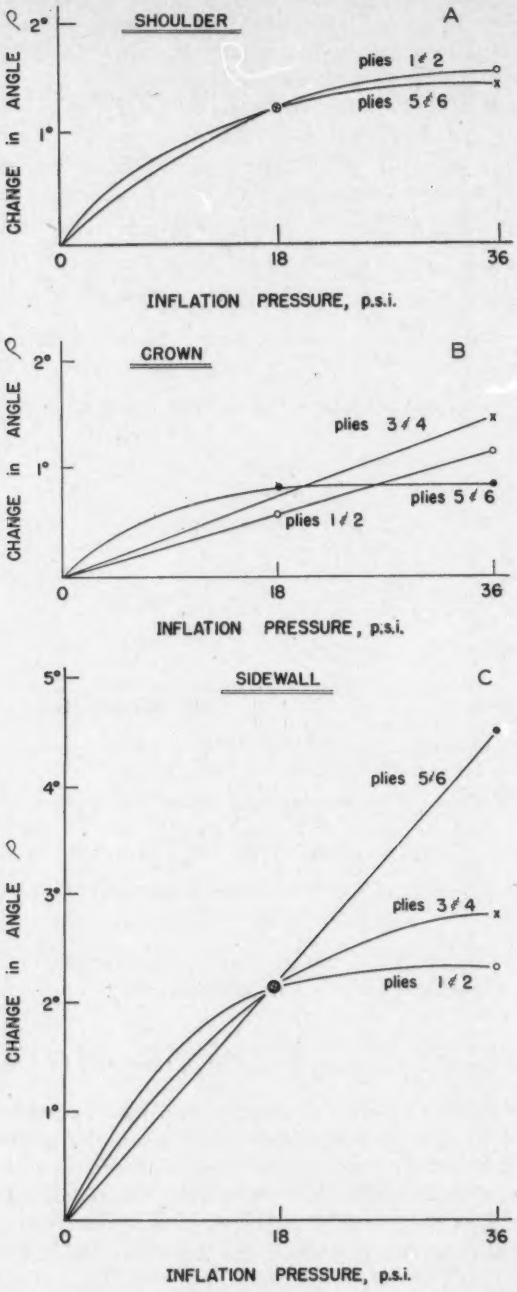

FIG. 10–A, B, C. *The change in angle between cords produced by the inflation of the tire.*

considerably less at a 12% deflection. Measurements were made only for plies 5 and 6. Since most separation failures occur there, it is likely that changes between any other pair of plies would be smaller.

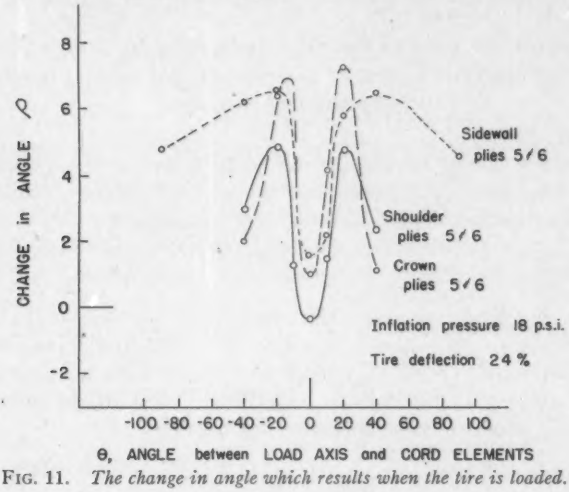

FIG. 11. *The change in angle which results when the tire is loaded.*

Summary

Data have been presented to show that when a tire is inflated its cords are elongated by about 2% and the angle between cords in adjacent plies change by about 1°. Differences between sidewall and crown are small. When the tire is loaded, there is very little increase in strain but rather a pronounced decrease in the loaded area. A similar effect results when the tire is subjected to a lateral thrust. It can be shown that this tension relief is related to the mechanism of tire load support in a direct analogy to a bicycle wheel.

Further changes produced by tire load can be seen in the change in angle between cords of adjacent plies. These, like the tension relief, reach their maximum value about 20° from the load axis. They are indicative of shear stresses in the rubber and are measures of the forces which produce separation between plies of a tire in service.

When the deflection of the tire is high, wrinkles develop in the tire sidewall at the place where cord compression (and angle change) are at their maximum value. Though not specifically discussed in this paper the tendency to wrinkle is intimately related to the quantity called stability.

The technique described should be useful to the design engineer in development of new tires though the trends shown may be helpful without more data. Strain ranges have been indicated which are of real value to those concerned with testing the component parts of the finished product. It is hoped that the tire user will be able to benefit from a knowledge of the strains produced in actual service. Here some caution will have to be exercised, for much of the data presented are for exaggerated deflection conditions.

NOTES

A Note on the Fate of Betaine Hydrochloride in Media Used for the Production of 2,3-Butanediol by Strains of *Bacillus polymyxa**

During production of 2,3-butanediol by fermentation of sugar-beet molasses in these laboratories, it was found that the molasses in use contained 4.8% by weight of betaine hydrochloride when analyzed by the method of Blood and Cranfield (2). It was therefore decided to investigate the effect and the fate of betaine hydrochloride in this fermentation, using *Bacillus polymyxa*.

Survey of the literature shows that little work has been done on the effect of betaine on microorganisms. Claasen (4) found that it was only slightly assimilated by yeast. Sumi (7) reported that the spores of *Aspergillus oryzeae* contained betaine in abundance. Dyer and Wood (5) found that various bacteria could produce methylamine from betaine.

A sugar-beet molasses and a yeast extract medium were used, and most of the work was conducted with *Bacillus polymyxa*, C3 (2). This strain normally gives a high yield of 2,3-butanediol in shaken culture. At the end of the fermentation the medium was made up to the original volume and an aliquot of the yeast extract medium was centrifuged to remove added calcium carbonate. The clear solution was used for analysis for 2,3-butanediol, as described by Leslie and Castagne (6). Ethanol was sometimes estimated, but in general it was found that the diol–ethanol ratio remained relatively constant.

The molasses medium, prepared by diluting sugar-beet molasses to one-tenth with tap water and adjusting the pH to 5.8 with acetic acid, contained 0.50% of betaine hydrochloride and 0.03% of trimethylamine. Analyses on the third, fourth, and fifth days of fermentation showed that neither of these compounds was being used by the organism. A similar experiment was conducted in which 0.48% of betaine hydrochloride was added to flasks containing yeast extract medium, originally containing 0.05% of betaine or betainelike substances. After a three-day fermentation they contained 0.50% of betaine hydrochloride. This difference is within the limits of error of measurement and it is clear that the added betaine hydrochloride was not acted on during the fermentation.

With the yeast extract medium, on the other hand, the addition of betaine hydrochloride gave a most marked increase in butanediol production with strain C3(2) of *Bacillus polymyxa*, as shown in Table I. Of 60 strains of *Bacillus polymyxa* tested, 52 showed a significant increase in yield of butanediol when betaine hydrochloride was added to the medium.

* *Issued as N.R.C. No. 2256.*

However, the addition of betaine (the free base) to the medium did not increase the yield of butanediol over that obtained in the controls.

When the hydrochloride was added to the yeast extract medium the pH fell to 2.75. Table I also brings out that the apparent beneficial effect of the added betaine hydrochloride was due to the acid part of the molecule.

TABLE I

THE EFFECT ON THE YIELD OF BUTANEDIOL AND ETHANOL BY *Bacillus polymyxa* C3 (2) WHEN BETAINE HYDROCHLORIDE IS ADDED TO A YEAST EXTRACT MEDIUM, AND WHEN THE pH IS BROUGHT TO 2.75 WITH ACID BEFORE STERILIZATION*

(Conditions—flasks shaken 100 times per minute for 72 hr. at 35°C.)

Conditions	Diol, %	Ethanol, %
Control	0.90	0.38
0.48% betaine hydrochloride added (pH 2.75)	1.59	0.50
pH brought to 2.75 with hydrochloric acid	1.59	
pH brought to 2.75 with hydrochloric acid and 0.23 gm. betaine (free base) added before inoculation	1.52	

* *Composition of medium. Difco Yeast Extract, 0.5 gm.; potassium dihydrogen phosphate, 0.05 gm.; dipotassium hydrogen phosphate, 0.05 gm.; magnesium sulphate heptahydrate, 0.02 gm.; reagent grade sucrose, 5 gm.; and distilled water to 100 ml.*
Two grams of sterile calcium carbonate was added to each flask before inoculation.

The most likely explanation is that during autoclaving the acid hydrolyzed the protein constituents of the yeast extract, making them more available to the organism. Block and Bolling (1) found that yeast protein on hydrolysis gave a balanced mixture of essential amino acids. Chatterjee (3) has suggested that the activity of yeast autolysate in growing various organisms was due to the presence of the sulphhydryl group. The apparent effect of betaine hydrochloride on the fermentation points to the need of care in interpreting experimental results.

1. BLOCK, R. J. and BOLLING, D. Arch. Biochem. 3: 217-226. 1943.
2. BLOOD, J. W. and CRANFIELD, H. J. Analyst, 61: 829-837. 1936.
3. CHATTERJEE, N. P. Calcutta Med. J. 39: 365-372. 1942.
4. CLAASEN, H. Z. angew. Chem. 39: 880-883. 1926.
5. DYER, F. E. and WOOD, A. J. J. Fisheries Research Board. Can. 7: 17-21. 1947.
6. LESLIE, J. D. and CASTAGNE, A. Can. J. Research, F, 24: 311-319. 1946.
7. SUMI, M. Biochem. Z. 195: 161-174. 1928.

RECEIVED JULY 26, 1950.
DIARMUID MURPHY
DIVISION OF APPLIED BIOLOGY,
NATIONAL RESEARCH LABORATORIES,
OTTAWA, CANADA.

SECTION F

INDEX TO VOLUME 28

Authors

Adams, A. M.—A comparative study of ascospore formation by 43 yeast cultures, 413.

Allan, A. B.—See King, R. O.

Allman, A. H.—See Lemon, H. W.

Anderson, J. A.—See Levi, I.

Arnell, J. C. and Henneberry, G. O.—Leveling bulb control for null manometers, 361.

Baerg, A., Klassen, J., and Gishler, P. E.—Heat transfer in a fluidized solids bed, 287.

Barnard, K. N.—Use of magnesium alloy anodes in ship protection, 417.

Barrington, M. G., Fleet, D. E., and McLaughlin, R. R.—The water repellency of silicone-treated sintered glass plates, 461.

Barton, G. M.—See Clark, R. H.

Bayley, C. H.—See Weatherburn, A. S.

Benson, B. and Larose, P.—The effect of sodium sulphate on the absorption of acid dyes by wool, 238.

Black, S. A.—See Mooney, M.

Brown, H. J. and Gibbons, N. E.—Enterococci as an index to fecal contamination in egg products, 107.

Clark, R. H., MacLean, H., Henderson, N., Barton, G. M., Johnson, A. L., Grunland, J. T., and Reynolds, W. L.—The emulsion polymerization of isoprene, 351.

Clendenning, K. A. and Wright, D. E.—Separation of starch and gluten. V. Problems in wheat starch manufacture arising from flour pentosans, 390.

Davies, J. M.—See Loughborough, D. L.

Dick, J. and Pugsley, L. I.—The arsenic, lead, tin, copper, and iron content of canned clams, oysters, crabs, lobsters, and shrimps, 199.

Durand, E. J.—See King, R. O.

Ellis, O. W.—See Kuhn, W. E.

Fleet, D. E.—See Barrington, M. G.

Gibbons, N. E.—Preservation of eggs. VII. Effect of age of egg and carbon dioxide content at time of oiling on keeping quality, 118.

——— and Rose, D.—Effect of *ante-mortem* treatment of pigs on the quality of Wiltshire bacon, 438.
See Brown, H. J.; Rose, D.

Gishler, P. E.—See Baerg, A.; Peterson, W. S.

Grace, N. H., Lips, H. J., and Zuckerman, A.—Canadian erucic acid oils. V. Physical, chemical, and edible properties of oil from weed seed screenings, 401.

Grunland, J. T.—See Clark, R. H.

Ham, M. P. and Smith, M. D.—Fluoride studies related to the human diet, 227.

Henderson, N.—See Clark, R. H.

Henneberry, G. O.—See Arnell, J. C.

Johnson, A. L.—See Clark, R. H.

King, R. O., Durand, E. J., and Allan, A. B.—The oxidation, decomposition, ignition, and detonation of fuel vapors and gases. XV. The concentration of finely divided carbon in town gas–air mixtures required to induce severe knocking combustion, 177. XVI. Benzene as a knocking fuel in conditions promoting the formation of finely divided carbon, 308.

————, ————, Wood, B. D., and Allan, A. B.—The oxidation, ignition, and detonation of fuel vapors and gases. XIII. The 12:1 compression ratio performance of the C.F.R. spark ignition engine using town gas; comparison with Diesel engines, 134. XIV. The cause of the effect of hydrogen sulphide to reduce the compression ratios at which fuel gases can be used in spark ignition engines, 166.

Klassen, J.—See Baerg, A.

Knapp, R. M.—See Lemon, H. W.

Kuhn, W. E. and Ellis, O. W.—A horizontal high vacuum electrical resistance furnace, 1.

Larose, P.—See Benson, B.

Lemon, H. W., Knapp, R. M., and Allman, A. H.—The effect of citric acid upon the oxidation of peanut oil and of the methyl esters derived from peanut oil, 453.

Levi, I. and Anderson, J. A.—Variations in protein contents of plants, heads, spikelets, and individual kernels of wheat, 71.

Lips, H. J.—Antioxidant properties of an ethylene dichloride extract of cottonseed meal, 451. Further studies on Canadian lard. Phosphorus content, color, and stability, 21.

————, and McFarlane, W. D.—Evaluation of antioxidant activity by stabilization of vitamin A and carotene, 157.
See Grace, N. H.

Lipsett, F.—The measurement of the heat output from radiant heating panels on a laboratory scale, 257.

Loughborough, D. L., Davies, J. M., and Monfore, G. E.—The measurements of strains in tires, 490.

Lusena, C. V.—Humin formation during wheat gluten hydrolysis, 234.

MacDougall, D. and Roadhouse, F. E.—A modified brucine method for the determination of nitrate, 280.

McFarlane, W. D.—See Lips, H. J.

McLaughlin, R. R.—See Barrington, M. G.

MacLean, H.—See Clark, R. H.

Marshall, H. B.—See Sitch, D. A.

Middleton, W. E. K.—The curve computer, 324.

Miles, B. J.—See Robinson, A. D.

Monfore, G. E.—See Loughborough, D. L.

Mooney, M. and Black, S. A.—Elongation hysteresis of *Hevea* and synthetic elastomers, 83.

Murphy, D.—A note on the fate of betaine hydrochloride in media used for the production of 2,3-butanediol by strains of *Bacillus polymyxa*, 502.

Newbould, F. H. S.—A simple spinner for roll tubes used in estimating bacterial populations, 464.

Peterson, W. S. and Gishler, P. E.—A small fluidized solids pilot plant for the direct distillation of oil from Alberta bituminous sands, 62.

Pfalzner, P. M.—On the friction of various synthetic and natural rubbers on ice, 468.

Potts, B. R.—Polarographic determination of titanium in paint pigments, 128.

Pugsley, L. I.—See Dick, J.

Reynolds, W. L.—See Clark, R. H.

Roadhouse, F. E.—See MacDougall, D.

Robinson, A. D., Tobias, C. H., and Miles, B. J.—The thiamine and riboflavin content of Manitoba grown wheat, oats, and barley of the 1947 crop, 341.

Rose, D. and Gibbons, N. E.—The effect of oiling and air cell mold on the oxygen and carbon dioxide contents of the air cells of eggs, 101.
See Gibbons, N. E.

Rose, G. R. F.—See Weatherburn, A. S.

Rose, R. C.—Extraction, fractionation, and evaluation of carrageenin, 202.

Sitch, D. A. and Marshall, H. B.—The effect of hemicelluloses on the papermaking properties of white birch, 376.

Smith, M. D.—See Ham, M. P.

Stedman, D. F.—Stress-strain curves for fatigued rubber, 31.

Tait, G. M. C.—Flowmeter error in measuring pulsating flow, 333.

Tobias, C. H.—See Robinson, A. D.

Weatherburn, A. S., Rose, G. R. F., and Bayley, C. H.—The sorption of soap by textile fibers, 51. The suspending power of detergent solutions. I. Pure soaps, 213. II. Soap-builder solutions, 363.

Webb, E. L. R.—A remote control projector for the examination of experimental records on 35 mm. film, 315.

Wood, B. D.—See King, R. O.

Wright, D. E.—See Clendenning, K. A.

Ziel, A. van der.—Noise suppression in triode amplifiers, 189.

Zuckerman, A.—See Grace, N. H.

SECTION F

INDEX TO VOLUME 28

Subjects

Absorption of acid dyes by wool, Effect of sodium sulphate on, 238.

Acid dyes, Absorption of, by wool, Effect of sodium sulphate on, 238.

Air–town gas mixtures, as fuel in spark ignition engines, Effect of carbon on, See Fuel vapors and gases.

Air cell mold, Effect of oiling and, on oxygen and carbon dioxide contents of air cells of eggs, 101.

Air cells of eggs, Effect of oiling and air cell mold on oxygen and carbon dioxide contents of, 101.

Alberta bituminous sands, A small fluidized solids pilot plant for direct distillation of oil from, 62.

Alloys, See Magnesium alloy anodes.

Amplifiers, See Triode Amplifiers.

Anodes, Magnesium alloy, Use of, in ship protection, 417.

Ante-mortem treatment of pigs, Effect of, on quality of Wiltshire bacon, 438.

Antioxidant activity, Evaluation of, by stabilization of vitamin A and carotene, 157.

Antioxidant properties of an ethylene dichloride extract of cottonseed meal, 451.

Arsenic, lead, tin, copper, and iron content of canned clams, oysters, crabs, lobsters, and shrimps, 199.

Ascospore formation by 43 yeast cultures, Comparative study of, 413.

Bacillus polymyxa, Fate of betaine hydrochloride in media used for production of 2,3-butanediol by strains of Bacillus polymyxa, 502.

Bacon, See Wiltshire bacon.

Bacterial populations, A simple spinner for roll tubes used in estimation of, 464.

Barley, oats, and wheat, Manitoba grown, 1947 crop, Thiamine and riboflavin content of, 341.

Benzene as a knocking fuel in spark ignition engines, See under Fuel vapors and gases.

Betaine hydrochloride, Fate of, in media used for the production of 2,3-butanediol by strains of Bacillus polymyxa, 502.

Birch, Papermaking properties of, Effect of hemicelluloses on, 376.

Bituminous sands, See Alberta bituminous sands.

Brucine method for determination of nitrate, Modified, 280.

2,3-Butanediol, Fate of betaine hydrochloride in media used for production of, by strains of Bacillus polymyxa, 502.

Canadian erucic acid oils, See Erucic acid oils.

Carbon, Effect of, in fuels for spark ignition engines, See under Fuel vapors and gases.

Carbon dioxide
and oxygen contents of air cells of eggs, Effect of oiling and air cell mold on, 101.
content of egg at time of oiling, Effect of age of egg and, on keeping quality, 118.

Carotene, Evaluation of antioxidant activity by stabilization of vitamin A and, 157.

Carrageenin, Extraction, fractionation, and evaluation of, 202.

Cell mold, See Air cell mold.

Cells, See Air cells of eggs.

C.F.R. spark ignition engine, The 12:1 compression ratio performance of, using town gas: comparison with Diesel engines, 134.
See also Fuel vapors and gases.

Clams, Arsenic, lead, tin, copper, and iron content of canned oysters, crabs, lobsters, shrimps, and, 199.

Color, phosphorus content, and stability of Canadian lard, 21.

Combustion, See Knocking combustion, under Fuel vapors and gases.

Compression ratios
(12:1) performance of the C.F.R. spark ignition engine using town gas; comparison with Diesel engines, 134.
at which fuel gas can be used in spark ignition engines, The cause of the effect of hydrogen sulphide to reduce, 166.

Computer, See Curve computer.

Contamination, See Fecal contamination.

Copper, iron, arsenic, lead, and tin content of canned clams, oysters, crabs, lobsters, and shrimps, 199.

Cottonseed meal, Antioxidant properties of an ethylene dichloride extract of, 451.

Crabs, Arsenic, lead, tin, copper, and iron content of canned clams, oysters, lobsters, shrimps, and, 199.

Cultures, See Yeast cultures.

Curve computer, 324.

Curves, Stress-strain, for fatigued rubber, 31.

Detergent solutions, Suspending power of, 213, 363.

Detonation, See under Fuel vapors and gases.

Diesel engines, The 12:1 compression ratio performance of the C.F.R. spark ignition engine using town gas; comparison with, 134.

Diet, Human, Fluoride studies related to the, 227.

Distillation, Direct, of oil from Alberta bituminous sands, A small fluidized solids pilot plant for, 62.

Dyes, See Acid dyes.

Egg products
Enterococci as an index of fecal contamination in, 107.
See also Eggs.

Eggs
Effect of oiling and air cell mold on the oxygen and carbon dioxide contents of air cells of, 101.
Preservation of,
VII. Effect of age of egg and carbon dioxide content at time of oiling on keeping quality, 118.
See also Egg products.

Elastomers, Synthetic, and *Hevea*, Elongation hysteresis of, 83.

Electrical resistance furnace, A horizontal high vacuum, 1.

Elongation hysteresis of *Hevea* and synthetic elastomers, 83.

Emulsion polymerization of isoprene, 351.

Engines, Internal combustion, Fuel tests with, See under Fuel vapors and gases.

Enterococci as an index of fecal contamination in egg products, 107.

Erucic acid oils, Canadian
V. Physical, chemical, and edible properties of oil from weed seed screenings, 401.

Ethylene dichloride extract of cottonseed meal, Antioxidant properties of, 451.

Evaluation of carrageenin, Extraction, fractionation, and, 202.

Extraction, fractionation, and evaluation of carrageenin, 202.

Fatigued rubber, Stress-strain curves for, 31.

Fecal contamination, in egg products, Enterococci as an index of, 107.

Film, 35 mm., A remote control projector for the examination of experimental records on, 315.

Flour pentosans, Problems in wheat starch manufacture arising from, 390.

Flow, Pulsating, Flowmeter error in measuring, 333.

Flowmeters, Error in measuring pulsating flow, 333.

Fluidized solids bed, Heat transfer in, 287.

Fluidized solids pilot plant for direct distillation of oil from Alberta bituminous sands, Small, 62.

Fluorides, Studies of, related to the human diet, 227.

Fractionation and evaluation of carrageenin, Extraction and, 202.

Friction of various synthetic and natural rubbers on ice, 468.

Fuel vapors and gases, Oxidation, ignition, and detonation of, XIII. The 12:1 compression ratio performance of the C.F.R. spark ignition engine using town gas; comparison with Diesel engines, 134.
XIV. The cause of the effect of hydrogen sulphide to reduce the compression ratios at which fuel gases can be used in spark ignition engines, 166.

XV. The concentration of finely divided carbon in town gas – air mixtures required to reduce severe knocking combustion, 177.
XVI. Benzene as a knocking fuel in conditions promoting the formation of finely divided carbon, 308.

Furnace, A horizontal high vacuum electrical resistance, 1.

Gas–air mixtures as fuel in internal combustion engines, See under Fuel vapors and gases.

Gases, See Fuel vapors and gases.

Glass plates, See Sintered glass plates.

Gluten
and starch, Separation of, 390.
See also Wheat gluten.

Heads, spikelets, and individual kernels of wheat, Variations in protein contents of plants, 71.

Heat output from radiant heating panels on a laboratory scale, Mearurement of, 257.

Heating panels, See Radiant heating panels.

Heat transfer in a fluidized solids bed, 287.

Hemicelluloses, Effect of, on papermaking properties of white birch, 376.

Hevea and synthetic elastomers Elongation hysteresis of, 83.

High vacuum electrical resistance furnace, A horizontal, 1.

Horizontal high vacuum electrical resistance furnace, A, 1.

Human diet, Fluoride studies related to, 227.

Humin formation during wheat gluten hydrolysis, 234.

Hydrogen sulphide, The cause of the effect of, to reduce the compression ratios at which fuel gases can be used in spark ignition engines, 166.

Hydrolysis of wheat gluten, Humin formation during, 234.

Hysteresis, Elongation, lof *Hevea* and synthetic elastomers, 83.

Ice, Friction of synthetic and natural rubbers on, 468.

Ignition, See under Fuel vapors and gases.

Ignition engines, Spark, Fuel tests with, See under Fuel vapors and gases.

Iron, Arsenic, lead, tin, and copper content of canned clams, oysters, crabs, lobsters, and shrimps, 199.

Isoprene, Emulsion polymerization of, 351.

Keeping quality of egg, Effect of age of egg and carbon dioxide content at time of oiling on, 118.

Kernels, of wheat, Variations in protein contents of plants, heads, spikelets, and individual, 71.

Knocking combustion in spark ignition engines, See under Fuel vapors and gases.

Knocking fuels, See Benzene under Fuel vapors and gases.

Lard, Canadian, Phosphorus content, color, and stability of, 21.

Lead, tin, copper, iron, and arsenic content of canned clams, oysters, crabs, lobsters, and shrimps, 199.

Leveling bulb control for null manometers, 361.

Lobsters, Arsenic, lead, tin, copper, and iron content of canned clams, oysters, crabs, shrimps, and, 199.

Magnesium alloy anodes, Use of, in ship protection, 417.

Manitoba grown wheat, oats, and barley, 1947 crop, Thiamine and riboflavin content of, 341.

Manometers, Null, Leveling bulb for, 361.

Meal, See Cottonseed meal.

Media used for production of 2,3-butanediol by strains of Bacillus polymyxa, Fate of betaine hydrochloride in, 502.

Methyl esters derived from peanut oil, Effect of citric acid on the oxidation of peanut oil and, 453.

Molds, See Air cell mold.

Nitrate, Modified brucine method for determination of, 280.

Noise suppression in triode amplifiers, 189.

Null manometers, Leveling bulb for, 361.

Oats, barley, and wheat, Manitoba grown, 1947 crop, Thiamine and riboflavin content of, 341.

Oiling of eggs
Effect of, and of air cell mold, on the oxygen and carbon dioxide contents of the air cells of eggs, 101.
Effect of age of egg and carbon dioxide content at time of, on keeping quality, 118.

Oils
from Alberta bituminous sands, A small fluidized solids pilot plant for direct distillation of, 62.
See also Erucic acid oils, Oiling of eggs; Peanut oil.

Oxidation
of peanut oil and of the methyl esters derived from peanut oil, Effect of citric acid on, 453.
See also under Fuel vapors and gases.

Oxygen and carbon dioxide contents of air cells of eggs, Effect of oiling and air cell mold on, 101.

Oysters, Arsenic, lead, tin, copper, and iron content of canned clams, crabs, lobsters, shrimps, and, 199.

Paint pigments, Polarography of titanium in, 128.

Panels, See Radiant heating panels.

Papermaking properties of white birch, Effect of hemicelluloses on, 376.

Peanut oil and methyl esters derived from peanut oil, Effect of citric acid on the oxidation of, 453.

Pentosans, See Flour pentosans.

Phosphorus content, color, and stability of Canadian lard, 21.

Pigments, See Paint pigments.

Pigs, Effect of ante-mortem treatment of, on quality of Wiltshire bacon, 438.

Pilot plant, Small fluidized solids, for direct distillation of oil from Alberta bituminous sands, 62.

Plant, See Pilot plant.

Plants, heads, spikelets, and individual kernels of wheat, Variations in protein contents of, 71.

Plates, See Sintered glass plates.

Polarography of titanium in paint pigments, 128.

Polymerization, See Emulsion polymerization.

Populations, See Bacterial populations.

Projector, Remote control, for the examination of experimental records on 35 mm. film, 315.

Protection of ships, Use of magnesium alloy anodes in, 417.

Protein contents of plants, heads, spikelets, and individual kernels of wheat, Variations in, 71.

Pulsating flow, Flowmeter error in measuring, 333.

Quality of Wiltshire bacon, Effect of ante-mortem treatment of pigs on the, 438.

Radiant heating panels, Measurement of heat output from, on a laboratory scale, 257.

Remote control projector for the examination of experimental records on 35 mm. film, 315.

Resistance furnace, A horizontal high vacuum electrical, 1.

Riboflavin and thiamine content of Manitoba grown wheat, oats, and barley of 1947 crop, 341.

Roll tubes used in estimation of bacterial populations, A simple spinner for, 464.

Rubber
Fatigued, Stress-strain curves for, 31.
Synthetic and natural, Friction of, on ice, 468.
tires, Measurement of strains in, 490.

Sands, See Alberta bituminous sands.

Screenings, See Weed seed screenings.

Ship protection, Use of magnesium alloy anodes in, 417.

Shrimps, Arsenic, lead, tin, copper, and iron content of canned clams, oysters, crabs, lobsters, and, 199.

Silicone, Water-repellency of sintered glass plates treated with, 461.

Sintered glass plates, Water-repellency of silicone-treated, 461.

Soap
Pure, and soap-builder solutions, Suspending power of, 203, 363.
Sorption of, by textile fibers, 51.

Sodium sulphate, Effect of, on absorption of acid dyes by wool, 238.

Solids, Fluidized, Pilot plant, small, for direct distillation of oil from Alberta bituminous sands, 62.

Sorption of soap by textile fibers, 51.

Spark ignition engines
Benzene as a knocking fuel in conditions promoting formation of finely divided carbon in, 308.
The cause of the effect of hydrogen sulphide to reduce the compression ratios at which fuel gases can be used in, 166.
See C.F.R. spark ignition engine.

Spikelets, and individual kernels of wheat, Variations in protein contents of plants, heads, 71.

Spinner for roll tubes used in estimating bacterial populations, A simple, 464.

Stability, phosphorus content, and color of Canadian lard, 21.

Stabilization of vitamin A and carotene, Evaluation of antioxidant activity by, 157.

Starch and gluten, Separation of,
V. Problems in wheat starch manufacture arising from flour pentosans, 390.

Strain
in tires, Measurements of, 490.
–stress curves for fatigued rubber, 31.

Stress-strain curves for fatigued rubber, 31.

Suspending power of detergent solutions.
I. Pure soaps, 213.
II. Soap-builder solutions, 363.

Synthetic elastomers, See Elastomers, Synthetic.

Textile fibers, Sorption of soap by, 51.

Thiamine and riboflavin content of Manitoba grown wheat, oats, and barley of 1947 crop, 341.

Tin, copper, iron, arsenic, and lead content of canned clams, oysters, crabs, lobsters and shrimps, 199.

Tires, Measurements of strains in, 490.

Titanium in paint pigments, Polarography of, 128.

Town gas
The 12:1 compression ratio performance of the C.F.R. spark ignition engine using, and comparison with Diesel engines, 134.
—air mixtures, The concentration of finely divided carbon in, required to induce severe knocking combustion in spark ignition engines, 177.

Triode amplifiers, Noise suppression in, 189.

Tubes, See Roll tubes.

Vacuum electrical resistance furnace, A horizontal high, 1.

Vapors, See Fuel vapors and gases.

Vitamin A and carotene, Evaluation of antioxidant activity by stabilization of, 157.

Water-repellency of silicone-treated sintered glass plates, 461.

Weed seed screenings, Physical, chemical, and edible properties of oil from, 401.

Wheat
oats and barley, Manitoba grown, 1947 crop, Thiamine and riboflavin content of, 341.
Variations in protein contents of plants, heads, spikelets, and individual kernels of 71.

Wheat gluten hydrolysis, Humin formation during, 227.

Wheat starch manufacture, Problems arising from flour pentosans, 390.

White birch, Papermaking properties of, Effect of hemicelluloses on, 376.

Wiltshire bacon, Effect of ante-mortem treatment of pigs on quality of, 438.

Yeast cultures, Comparative study of ascospore formation by, 413.